The Morphometry of the Coregonine Fishes of Great Bear Lake, N.W.T.

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ABSTRACT

Morphometric measurements and counts were made on representative samples of 78 lake whitefish *Coregonus clupeaformis*, 77 ciscoes *Leucichthys artedi* and 8 round whitefish *Prosopium cylindraceum* from Great Bear Lake, N.W.T. The amount of variability found within this homogeneous population of whitefish casts doubt on the validity of some of the species of whitefish which have been described from this region. The same can be said of the ciscoes.

INTRODUCTION

During the summer of 1945 the author was one of a party of three sent by the Fisheries Research Board of Canada to investigate the fisheries potentialities of Great Bear Lake. Three species of coregonines were taken during the investigation: lake whitefish *Coregonus clupeaformis*, cisco *Leucichthys artedi*, and round whitefish *Prosopium cylindraceum*. As far as could be determined no other species of coregonines are present in the lake. Observations on the availability, distribution, rate of growth, size at maturity, sex ratio, and food of each species have been published (Kennedy, 1949).

It is a pleasure to acknowledge the assistance of Dr. R. B. Miller, who was in charge of the party, and of Mr. M. B. Smith, the third member, in getting the specimens used in this study.

Great Bear Lake lies in the Northwest Territories on the Arctic circle. It has a water area of about 11,800 square miles (4,750,000 hectares), with islands included about 12,000 square miles (4,800,000 hectares). Most of the lake is over 400 feet (120 metres) deep and the maximum recorded depth is 1,300 feet (400 metres). The main body of the lake is clear and cold with a meagre biota. The coregonines appear to be almost entirely confined to the periphery of the lake. Details on topography, limnology and related matter have been published (Miller, 1947).

MATERIAL AND METHODS

The fish used in this study were taken entirely by gill-nets of 2½-, 3-, 4-, 5-, and 5½-inch mesh (stretched measure). Several thousand small coregonines were taken in seine hauls but the data on the few that were measured are excluded.

Detailed measurements and counts were made on 78 lake whitefish *Coregonus clupeaformis*, 77 ciscoes *Leucichthys artedi* and 8 round whitefish *Prosopium cylindraceum*. Standard length, head length and depth, eye diameter, snout

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length, interorbital width, maxillary length, caudal peduncle length and depth, body width and depth, dorsal fin height and base, anal fin height and base, and pectoral and pelvic fin lengths were measured. Dorsal and anal fin rays, branchiostegal rays, scales, and gill rakers were counted. These measurements and counts were made according to the methods used by Dymond (1943). Vertebrae were also counted on some of the fish on which the measurements and other counts were taken, and on some others taken at random from the catches. All the vertebrae were counted including the urostyle.

Whenever possible the measurements and counts were made on fresh specimens, but 31 *Coregonus,* 49 *Leucichthys* and 3 *Prosopium* were preserved in formalin and measured later. Twelve *Leucichthys* and seven *Coregonus* which had been measured while fresh were preserved and were measured again with the others. The average observed shrinkage of each part of these individuals with preservation was used to calculate the probable original size of the part in each preserved fish. The factors by which measurements on preserved fish were multiplied to give the probable measurements of fresh fish are shown in Table I.

TABLE I. The factors by which measurements on fish from Great Bear Lake, that were preserved in 5 per cent formalin, were multiplied to give the probable fresh measurements.

Measurement	*Coregonus*	*Leucichthys*
Standard length	1.08	1.04
Head length	1.04	1.00
Head depth	1.00	1.00
Eye diameter	1.00	1.04
Snout length	1.08	1.05
Interorbital width	1.00	1.05
Maxillary length	1.00	1.00
Body depth	1.00	1.00
Body width	1.00	1.00
Caudal peduncle length	1.15	1.11
Caudal peduncle depth	1.00	1.00
Dorsal fin height	1.00	1.00
Dorsal fin base	1.00	1.00
Anal fin height	1.00	1.00
Anal fin base	1.06	1.00
Pectoral fin length	1.09	1.05
Pelvic fin length	1.05	1.06

Records of the original measurements are on file at the Central Fisheries Research Station, 165 Garry Street, Winnipeg, as are various graphs plotted between logarithms of body parts and logarithms of standard length. In the analyses that follow, mature *Coregonus* and *Leucichthys* are treated separately from the corresponding immature fish, since the above-mentioned graphs in many cases indicated a change in growth rate of the part relative to the whole at the time of maturity.

COREGONUS

The sample of whitefish on which detailed measurements and counts were made consisted of three subsamples which came from three widely separated parts of the lake: the west end of Smith Arm; the east shore of McTavish Arm; and various places in McVicar Arm. The sample is presumably representative of

the whitefish population in the lake, since the places where the subsamples were taken were the only places on the lake where whitefish were found or reported in any quantity, and since the numbers measured from each locality were approximately proportioned to the relative catches. Analysis of variance showed that the subsamples of mature fish differed significantly among themselves in average head length, head depth, snout length, interorbital width, caudal peduncle depth, pectoral fin length, and pelvic fin length for a given standard length, and that subsamples of immature fish differed significantly among themselves in caudal peduncle depth, body depth, body width and dorsal fin base. In no case was there a significant difference in the rate at which the size of a body part changed with an increase in standard length. Further investigation showed that Smith Arm and McVicar Arm fish tended to be alike, whereas those from McTavish Arm tended to differ from them. The divergent nature of the McTavish Arm fish seemed to be entirely responsible for the fact that the tests showed the above-mentioned differences to be significant.

The Smith Arm subsample was taken about 400 shoreline miles from where the McVicar Arm subsample was taken (200 miles by the most direct route across the "biological vacuum" of the lake) compared with about 100 shoreline miles between the places where the McTavish and McVicar Arms subsamples were taken. The subsamples from Smith and McVicar Arms were taken from similar habitats, namely at the blind ends of long narrow arms, where relatively large rivers produced considerable areas of water which were noticeably warmer than the open lake. Although the sample from McTavish Arm was also taken mostly from the vicinity of river mouths, the rivers influenced a much smaller surrounding area, and the cold water of the open lake was much closer. In other words, two groups of fish which were nearest to one another, but whose habitat differed, tended to be more different from one another than two groups which were farther apart, but which lived in more similar habitats. Martin (1949) has shown how fish which are the same genetically can have different average sizes of body parts for a given standard length (but generally the same rate of increase in the average size of the body part with standard length) as the result of continual exposure to different water temperatures. It is, therefore, assumed that the difference observed between the whitefish of Smith and McVicar Arms and those of McTavish Arm are correlated with differences in average water temperatures and do not represent genetic differences among the fish.

Fish from the three localities did not differ significantly among themselves in average number of vertebrae, dorsal fin rays, anal fin rays, branchiostegal rays, scales, and gill rakers.

The individual measurements and counts constitute a great mass of data, awkward to publish in its entirety, and probably incomprehensible in that form. Therefore, they have been summarized in Tables II, III, IV, V and VI. The disadvantage of such summaries is that only one body part is considered at a time, and any tendency for a particular fish to be unusual in several respects is not brought out. However, a careful examination of the original data shows that

TABLE II. The regression of various measurements of body parts on standard length, with their standard deviation, in a representative sample of 52 mature Great Bear Lake whitefish, and the values needed to test the significance of any apparent difference between this regression and others (X = log. 10 of standard length and Y = log. 10 of size of body part in millimetres).

Body part	Average size of the body part for a given standard length	Standard error of estimate	Quantities needed for comparisons					
			n	SX	SY	SX^2	SXY	SY^2
Head length	$Y = 0.97X - 0.61$	± 0.016	51	137.85	102.92	372.7729	278.3545	207.8730
Head depth	$Y = 0.96X - 0.75$	± 0.019	52	140.54	96.05	380.0090	259.7593	177.5929
Eye diameter	$Y = 0.55X - 0.19$	± 0.026	52	140.54	66.77	380.0090	180.5529	85.8197
Snout length	$Y = 1.16X - 1.63$	± 0.036	52	140.54	77.52	380.0090	209.7121	115.8578
Interorbital width	$Y = 1.15X - 1.63$	± 0.024	52	140.54	77.11	380.0090	208.6034	114.6030
Maxillary length	$Y = 1.26X - 1.90$	± 0.030	52	140.54	78.47	380.0090	212.2983	118.7351
Body depth	$Y = 1.04X - 0.68$	± 0.026	52	140.54	110.03	380.0090	297.5560	233.0387
Body width	$Y = 1.06X - 1.07$	± 0.035	52	140.54	93.18	380.0090	252.0466	167.2623
Caudal peduncle length	$Y = 0.91X - 0.77$	± 0.040	52	140.54	88.53	380.0090	239.4270	150.9485
Caudal peduncle depth	$Y = 0.90X - 0.83$	± 0.022	52	140.54	83.65	380.0090	226.2357	134.7279
Dorsal height	$Y = 0.74X - 0.11$	± 0.028	52	140.54	98.68	380.0090	266.8298	187.3990
Dorsal base	$Y = 0.97X - 0.86$	± 0.031	52	140.54	91.87	380.0090	248.4638	162.5201
Anal height	$Y = 0.89X - 0.63$	± 0.027	52	140.54	92.24	380.0090	249.4501	163.7930
Anal base	$Y = 0.91X - 0.73$	± 0.021	52	140.54	90.51	380.0090	244.7784	157.7058
Pectoral length	$Y = 0.91X - 0.52$	± 0.024	52	140.54	100.68	380.0090	272.2641	195.1036
Pelvic length	$Y = 0.87X - 0.43$	± 0.029	52	140.54	99.28	380.0090	268.4727	189.7188

those individuals which were noticeably divergent with respect to one characteristic were not necessarily divergent with respect to others.

Tables II and III deal with mature fish only. In the first column of Table II is a series of equations from each of which the average size of the respective body part for any given length can be derived. These equations are presumably applicable to all mature Great Bear Lake whitefish since the sample was, as far as can be determined, representative of that population. The second column indicates the respective variances of these averages, while the remaining columns give the information which would be required, should it ever become desirable, to test the significance of differences between this population and some other one for which similar values would be available.

TABLE III. The ranges within which 95 per cent of the proportionate measurements of body parts of mature Great Bear Lake whitefish are expected to fall, on the basis of the relationship between standard lengths and the sizes of the various body parts in a representative sample of 52.

Body part	Standard lengths in millimetres				
	300	400	500	600	700
Head length	192–229	192–225	191–223	190–222	189–222
Head depth	129–158	128–154	128–153	126–152	125–151
Eye diameter	42–55	37–48	34–43	31–40	29–37
Snout length	47–68	50–70	52–72	53–75	54–77
Interorbital width	49–63	52–65	54–67	55–69	56–71
Maxillary length	48–66	52–70	55–74	58–77	60–81
Body depth	221–292	226–291	229–293	230–295	230–298
Body width	99–144	103–143	104–145	105–147	105–149
Caudal peduncle length	84–130	84–124	83–121	81–119	80–119
Caudal peduncle depth	76–95	74–92	73–89	71–88	70–87
Dorsal height	154–208	145–190	138–179	131–171	125–165
Dorsal base	100–139	101–135	100–134	100–134	99–134
Anal height	108–144	106–137	104–134	102–131	99–130
Anal base	102–128	101–123	99–120	97–119	96–118
Pectoral length	158–204	156–196	153–192	150–189	148–187
Pelvic length	148–201	145–190	141–184	137–180	134–177

Table III indicates the range within which most of the proportionate parts of measurements on mature Great Bear Lake whitefish are expected to fall (where proportionate parts are defined as: measurement of the part ÷ standard length × 1000). Conversely any mature whitefish from whatever source whose measurements fall within this range cannot be considered to be morphologically different from typical Great Bear Lake fish. Table III was derived by calculating the average size of each body part and its variance at the indicated standard lengths, and then converting these values into proportional parts.

Tables IV and V are the same as Tables II and III respectively except that they deal with the immature fish in the sample. Table VI summarizes the counts of meristic characters on mature and immature fish combined. The various statistical analysis techniques used are all given in Snedecor (1946).

Dymond (1943) gives proportionate measurements for whitefish from several localities in northwestern Canada, also measurements on fish from Alaska which had been identified as *C. kennicotti* and *C. nelsoni* and on a specimen of

TABLE IV. The regressions of various measurements of body parts on standard length with their standard errors of estimate in a representative sample of 26 immature Great Bear Lake whitefish, and the values needed to test the significance of any apparent difference between this regression and others (X = log. 10 of stand ard lengthand Y = log. 10 of size of body part in millimetres).

Body part	Average size of the body part for a given standard length	Standard error of estimate	Quantities needed for comparisons					
			n	SX	SY	SX^2	SXY	SY^2
Head length	$Y = 0.89X - 0.42$	± 0.016	26	65.05	47.05	162.8899	117.8398	85.2591
Head depth	$Y = 0.95X - 0.74$	± 0.021	26	65.05	42.36	162.8899	106.1141	69.1508
Eye diameter	$Y = 0.58X - 0.31$	± 0.021	26	65.05	29.63	162.8899	74.2131	33.8247
Snout length	$Y = 1.06X - 1.42$	± 0.038	26	65.05	32.24	162.8899	80.8107	40.1710
Interorbital width	$Y = 1.07X - 1.44$	± 0.024	26	65.05	32.13	162.8899	80.5366	39.8797
Maxillary length	$Y = 0.89X - 0.96$	± 0.026	26	65.05	33.12	162.8899	82.9886	42.3176
Body depth	$Y = 1.27X - 1.30$	± 0.028	26	65.05	48.48	162.8899	121.4703	90.6392
Body width	$Y = 0.98X - 0.89$	± 0.043	26	65.05	40.78	162.8899	102.1660	64.1418
Caudal peduncle length	$Y = 1.03X - 1.04$	± 0.033	26	65.05	39.91	162.8899	99.9955	61.4355
Caudal peduncle depth	$Y = 0.99X - 1.07$	± 0.021	26	65.05	36.64	162.8899	91.8088	51.7804
Dorsal height	$Y = 0.80X - 0.27$	± 0.035	25	62.61	43.24	156.9363	108.3989	74.9024
Dorsal base	$Y = 1.22X - 1.50$	± 0.035	26	65.05	40.30	162.8899	100.9978	62.7026
Anal height	$Y = 0.91X - 0.72$	± 0.030	26	65.05	40.57	162.8899	101.6304	63.4431
Anal base	$Y = 1.02X - 1.02$	± 0.038	26	65.05	39.58	162.8899	99.1683	60.4328
Pectoral length	$Y = 0.97X - 0.73$	± 0.024	25	62.61	42.61	156.9363	106.8446	72.7667
Pelvic length	$Y = 0.90X - 0.57$	± 0.024	26	65.05	44.05	162.8899	110.3360	74.7589

TABLE V. The ranges within which 95 per cent of the proportionate measurements of body parts of immature Great Bear Lake whitefish are expected to fall on the basis of the relationships between standard lengths and the sizes of various body parts in a representative sample of 26.

Body part	Standard length in millimetres			
	200	300	400	500
Head length	196–233	189–221	183–214	177–211
Head depth	122–154	121–149	119–147	117–147
Eye diameter	47–59	40–49	35–44	32–40
Snout length	43–66	45–66	46–67	46–69
Interorbital width	46–60	48–61	49–62	49–64
Maxillary length	54–72	52–68	51–66	49–65
Body depth	175–237	199–260	213–282	224–303
Body width	93–149	93–144	94–144	91–146
Caudal peduncle length	89–128	92–126	92–128	91–131
Caudal peduncle depth	73–90	73–89	73–89	72–90
Dorsal height	152–223	144–201	135–191	127–186
Dorsal base	83–123	93–131	98–140	102–150
Anal height	101–141	99–133	96–131	93–130
Anal base	84–128	87–126	87–127	86–130
Pectoral length	140–184	141–179	139–178	139–177
Pelvic length	143–186	139–176	135–172	131–170

C. nasus from Siberia. A comparison of the values in Tables III, V and VI with Dymond's Table I shows that, considering the effects of preservation, neither *Coregonus clupeaformis* from other parts of northwestern Canada, nor the specimens of *C. nelsoni* used can be regarded as different from typical Great Bear Lake *Coregonus*; and further that the specimens of *C. kennicotti* and *C. nasus* are not strikingly different. In view of the amount of difference which Martin (1949) showed can result from environmental differences, the possibility that all four species are synonomous cannot be disregarded. However, since only a few specimens are available for comparison, and since the amount that they have changed with preservation is not exactly known, further discussion on this subject seems unwarranted.

TABLE VI. A summary of counts on body parts of whitefish from Great Bear Lake, 1945.

Body part	Number counted	Range in count	Average count	Standard deviation
Vertebrae	49	59–64	61.8	± 1.1
Dorsal rays	78	12–16	13.7	± 0.8
Anal rays	78	12–16	13.8	± 1.0
Branchiostegals	78	8–10	9.2	± 0.5
Scales	57	72–85	77.8	± 3.1
Gill rakers	77	23–31	27.0	± 1.5

LEUCICHTHYS

Detailed measurements and counts were made on ciscoes from two parts of the lake, namely from the east shore of McTavish Arm (mostly from Echo Bay) and from Franklin Bay at the west end of Keith Arm. Almost all of the ciscoes caught during the survey came from one of these two localities, and the size of the subsamples from each place corresponded roughly to the apparent relative

TABLE VII. The regression of various measurements of body parts on standard length with their standard deviations in a representative sample of 77 mature Great Bear Lake ciscoes, and the values needed to test the significance of any apparent difference between this regression and others (X = log. 10 of standard length and Y = log. 10 of size of body part in millimetres).

Body part	Average size of the body part for a given standard length	Standard error of estimate	Quantities needed for comparisons					
			n	SX	SY	SX^2	SXY	SY^2
Head length	$Y = 0.89X - 0.41$	± 0.015	77	190.36	137.69	470.7036	340.4818	246.3051
Head depth	$Y = 0.95X - 0.73$	± 0.019	77	190.36	123.81	470.7036	306.1728	199.1869
Eye diameter	$Y = 0.57X - 0.27$	± 0.027	77	190.36	87.81	470.7036	217.1383	100.2217
Snout length	$Y = 1.01X - 1.34$	± 0.026	77	190.36	89.18	470.7036	220.5667	103.4324
Interorbital width	$Y = 0.87X - 0.92$	± 0.028	77	190.36	95.40	470.7036	235.9309	118.3296
Maxillary length	$Y = 0.86X - 0.80$	± 0.020	77	190.36	101.69	470.7036	251.4797	134.3967
Body depth	$Y = 1.26X - 1.30$	± 0.026	77	190.36	140.48	470.7036	347.4147	256.4942
Body width	$Y = 1.10X - 1.15$	± 0.033	77	190.36	119.91	470.7036	296.5456	186.9287
Caudal peduncle length	$Y = 0.92X - 0.75$	± 0.038	76	187.88	116.54	464.5532	288.1859	178.8932
Caudal peduncle depth	$Y = 0.88X - 0.83$	± 0.026	76	187.92	101.96	464.7500	252.1913	136.9076
Dorsal height	$Y = 0.83X - 0.44$	± 0.022	77	190.36	125.04	470.7036	309.2033	203.1527
Dorsal base	$Y = 1.11X - 1.25$	± 0.038	77	190.36	114.87	470.7036	284.0868	171.5895
Anal height	$Y = 1.00X - 1.03$	± 0.031	77	190.36	110.80	470.7036	274.0148	159.6010
Anal base	$Y = 1.20X - 1.53$	± 0.031	77	190.36	110.96	470.7036	274.4294	160.1082
Pectoral length	$Y = 0.95X - 0.73$	± 0.028	76	187.89	123.32	464.6027	304.9660	200.2462
Pelvic length	$Y = 0.86X - 0.52$	± 0.024	77	190.36	124.22	470.7036	307.1790	200.5098

abundance. No other localities are known in which there are appreciable concentrations of them.

The analysis of variance was used to compare mature males from Keith Arm with mature females and to compare all mature Keith Arm fish with all mature McTavish Arm fish regardless of sex. The tests showed no significant difference between the sexes in the average size of any body part for a given standard length. Also there was no significant difference between the sexes in the rate at which the average size of any body part changed with a change in standard length. The two sexes of these fish are seen to be remarkably similar morphologically. The mature fish from the two localities differed significantly in the average size of several body parts for a given standard length. The ciscoes from McTavish Arm had significantly longer and deeper heads, longer maxillaries, longer and deeper caudal peduncles, deeper and wider bodies, and longer dorsal, anal, pectoral and pelvic fins for a given standard length than did the Keith Arm fish. They did not differ significantly from one another in the rate at which any body part increased with an increase in body length. As in the case of the whitefish it seems likely that the slight differences between the two subsamples are associated with environmental differences. The average temperature of Franklin Bay is slightly higher than that of the east end of McTavish Arm.

Eleven immature ciscoes from Keith Arm and eleven from McTavish Arm were available. However, there was such a difference between the ranges in length of the two subsamples (Keith Arm 250 millimetres to 295 millimetres; McTavish Arm 40 millimetres to 65 millimetres) that it was not thought advisable to compare them.

Ciscoes from Keith Arm had an average of 59.5 vertebrae (71 fish) compared with an average of 58.7 vertebrae (19 fish) in ciscoes from McTavish Arm. The two averages are significantly different. It is assumed that this differ-

TABLE VIII. The ranges within which 95 per cent of the proportionate measurements of body parts of mature Great Bear Lake ciscoes are expected to fall on the basis of the relationship between standard lengths and the size of various body parts in a representative sample of 77.

Body part	Standard length in millimetres		
	200	300	400
Head length	200–234	193–221	186–215
Head depth	127–154	125–149	122–147
Eye diameter	48–64	41–52	36–47
Snout length	42–55	43–55	43–55
Interorbital width	53–71	51–67	49–65
Maxillary length	67–83	64–77	61–75
Body depth	177–232	200–254	214–277
Body width	98–140	104–142	106–148
Caudal peduncle length	97–145	96–137	93–136
Caudal peduncle depth	68–89	66–83	63–81
Dorsal height	135–170	128–156	121–150
Dorsal base	82–123	88–125	89–131
Anal height	79–109	80–107	79–108
Anal base	73–101	81–108	84–116
Pectoral length	124–167	124–161	121–160
Pelvic length	129–165	124–154	118–149

ence is related to the temperature differences. Fish from the two localities did not differ significantly in average counts of dorsal or anal fin rays, branchiostegal rays, scales, or gill rakers.

Tables VII and VIII summarize the measurement data for all the mature Great Bear Lake ciscoes measured regardless of the particular locality from which they were taken. They correspond to Tables II and III. The measurement data on immature ciscoes are not presented here. As in the case of whitefish a careful examination of the original data showed that those individuals which were noticeably divergent with respect to one characteristic were not necessarily divergent with respect to others. Table IX summarizes the counts on these mature ciscoes as well as on the eleven largest immature ciscoes. Note that in the case of a few randomly chosen fish, vertebrae were counted where no other measurements or counts were made. Few scale counts were possible because scales were easily lost in handling.

TABLE IX. A summary of counts of body parts of ciscoes from Great Bear Lake, 1945.

Body part	Number counted	Range in count	Average count	Standard deviation
Vertebrae	90	56–62	59.4	± 1.1
Dorsal rays	88	12–15	13.3	± 0.7
Anal rays	88	12–15	13.5	± 0.8
Branchiostegals	88	7–10	8.5	± 0.6
Scales	18	73–84	78.6	± 2.8
Gill rakers	87	41–50	45.0	± 2.0

TABLE X. Proportionate measurements and counts of body parts of 8 fresh *Prosopium* taken in Great Bear Lake, 1945.

Body part	Fish number							
	1	2	3	4	5	6	7	8
Standard length	251	251	253	267	278	280	298	305
Head length	191	187	178	202	190	189	188	197
Head depth	120	123	107	127	126	122	121	125
Eye diameter	40	42	40	43	41	39	40	39
Snout length	46	44	43	49	50	43	45	46
Interorbital width	52	50	47	58	52	50	54	54
Maxillary length	42	42	38	45	43	39	42	46
Body depth	179	198	170	187	205	179	187	180
Body width	131	128	130	135	126	111	128	124
Caudal peduncle length	107	139	134	135	133	125	131	122
Caudal peduncle depth	76	72	67	73	72	73	67	67
Dorsal height	139	131	134	142	137	140	131	133
Dorsal base	131	123	134	124	119	122	121	118
Anal height	104	92	103	112	—	100	104	108
Anal base	92	100	87	90	90	82	87	85
Pectoral length	139	135	123	150	140	136	131	138
Pelvic length	115	115	107	124	119	118	114	124
Dorsal rays	15	14	15	14	14	15	15	13
Anal rays	13	11	11	12	12	13	12	12
Branchiostegals	7	7	7	7	7	8	7	7
Scales	86	87	102	—	—	—	—	90
Gill rakers	21	21	18	17	20	20	18	20

When the effects of preservation are taken into account a comparison of these data with comparable measurements and counts given by Dymond (1943) tends to confirm his opinion that the ciscoes of Great Bear Lake are *Leucichthys artedi*—of which *L. lucidus* is therefore a synonym. The slight disagreement with Dymond on fin-ray counts of Great Bear Lake ciscoes probably represents a difference of opinion as to what constitutes a fin ray. The degree to which some characters vary within the single species represented by the data in Tables VII, VIII and IX suggests that further investigation may reduce other species of *Leucichthys* to synonymy.

PROSOPIUM

Since the sample of *Prosopium* was small, it was not treated like the samples of whitefish and ciscoes; instead proportionate measurements of each individual were calculated, which are shown in Table X along with the counts.

Dymond (1943) gave the averages and ranges of proportionate measurements of four *Prosopium cylindraceum* from Great Bear Lake. Considering the effects of preservation the measurements and counts given in Table X do not differ appreciably from those given by Dymond.

SUMMARY

The objective of this paper has been to make available certain morphometric data on the coregonine fishes of Great Bear Lake. The data summarized here more than doubles the amount that is available for all of northwestern Canada.

Because of the paucity of comparable material from this region few comparisons are possible. However, the available data do serve to confirm Dymond's (1943) conclusions that certain previously recognized species should be reduced to synonymy, and in addition they suggest that some of the species of Canada, Alaska and Siberia which Dymond recognized may be reduced to synonymy when more data are available.

REFERENCES

Dymond, J. R. The coregonine fishes of Northwestern Canada. *Trans. Roy. Canadian Inst.*, **24**(II), 171–231, 1943.

Kennedy, W. A. Some observations on the coregonine fish of Great Bear lake, N.W.T. *Bull. Fish. Res. Bd. Canada*, No. 82, 1949.

Martin, W. R. The mechanics of environmental control of body form in fishes. *Univ. Toronto Studies, Biol. Series*, No. 58 (*Pub. Ontario Fish. Res. Lab.*, No. 70), 1949.

Miller, R. B. North West Canadian fisheries surveys in 1944–45. IV. Great Bear Lake. *Bull. Fish. Res. Bd. Canada*, No. 72, 1947.

Snedecor, G. W. Statistical methods applied to experiments in agriculture and biology. Ames, Iowa: Collegiate Press, 1946.

The Effect of Fasting on the Bacterial Flora of the Intestine of Fish[1]

By Leo Margolis[2]

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ABSTRACT

Sixteen bullheads (*Ameiurus nebulosus*) and three speckled trout (*Salvelinus fontinalis*) were kept in complete starvation for periods of time varying from one to two months and seven to ten days respectively. They were then examined, by culture methods, for the presence of bacteria in the intestine. Fifteen bullheads and the three trout did not yield bacteria. A Pseudomonad was obtained, in small numbers, from the intestine of one bullhead. Three bullheads and two trout examined two hours after the last feeding yielded intestinal bacteria in large numbers. In non-feeding fish, bacteria are usually absent from the intestine.

INTRODUCTION

General opinion today holds that there are no common bacterial commensals in the intestine of fish, but that the presence of a bacterial flora depends solely upon the recent intake of food and the degree of contamination of the food and water.

Obst (4) observed that of 94 herring (*Clupeus harengus*) taken off the coast of Maine, with no visible food in the alimentary tract, only 13 showed the presence of bacteria in the stomach or intestine. In this paper he mentions that Gillepsie (2) in 1898 in Scotland, had noted that the gut of certain fishes was free from bacteria in the absence of food. Hunter (3) found the stomachs and intestines of spawning salmon to be sterile. Salmon returning to fresh water to spawn do not feed and hence the digestive tract is free from food. Blake (1) examined 22 salmonid fishes, in which there was apparently no food in the digestive tract. In 15 of these, either the intestine or the stomach or both were free from bacteria. Of the remaining seven fish, Blake believed that four had only very shortly before examination digested the last of its food.

METHODS

With the above results in mind it was decided to keep fish in troughs without feeding them and observe the effects on the micro-flora of the gut. The troughs were supplied with an ample supply of running well-water.

Sixteen common bullheads (*Ameiurus nebulosus*) and three speckled trout (*Salvelinus fontinalis*) were used in the experiment. The bullheads had not been fed for from one to two months and the trout for from seven to ten days prior to making bacterial cultures of the intestinal contents. As there was no food present in the gut, the samples consisted of mucus, which was always present.

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Before subjecting the experimental fish to fasting, three bullheads and two trouts were examined for intestinal bacteria, two hours after their last feeding. In these fish food was present in the intestine.

The fish were opened aseptically and the intestine, after searing the surface, was slit in the anterior, middle and posterior one-third regions. Samples of the intestinal contents were obtained by inserting a sterile bacteriological wire loop through the slits in the intestine. Two loopfuls of material from each of the intestinal regions sampled (one loopful was used in the cases where the fish had eaten shortly before examination) were inoculated into each of two nutrient broth (Difco) tubes and plated on each of two rabbit blood agar plates. One broth tube and one blood agar plate of each set were incubated aerobically, while the other tube and plate were incubated anaerobically in a Brewer jar. Both sets were incubated at room temperature (20°–25°C.). The culture tubes and plates were observed for three weeks, at which time, if there was no visible bacterial growth, either anaerobically or aerobically, the intestinal region from which the samples had been taken was proclaimed free from bacteria.

RESULTS

Bacterial growth was obtained in all the broth and on all the plate cultures of intestinal contents of the fish which had eaten two hours previous to being examined. Two to four hundred colonies appeared on each plate.

Of the 16 non-feeding bullheads, 15 were free of intestinal bacteria, as were also the three non-feeding trout. Cultures of the contents of the intestine of the sixteenth bullhead showed the presence of a Pseudomonad. This organism was present in pure culture in both the aerobic and anaerobic broth and agar plate cultures. The number of colonies appearing on the plates varied from 15 to 32, a comparatively small number in contrast to the number of colonies that appeared on culture plates of a similar sample from an actively feeding fish.

These results indicate that bacteria do not usually persist in the intestine of fasting fish.

BIBLIOGRAPHY

1. Blake, I. Some observations on the bacterial flora of the alimentary tract of certain Salmonidae. Final Report of the Furunculosis Committee, App. B, pp. 60–67. H.M. Stationery Office, Edinburgh, 1935.
2. Gillepsie, A. L. Fishery Board for Scotland, Report for 1898, p. 23. (Quoted by Obst (4).)
3. Hunter, A. C. Bacterial decomposition of salmon. *J. Bacteriol.* **5**, 353–358, 1920.
4. Obst, M. M. A bacteriologic study of sardines. *J. Infectious Diseases*, **24**, 158–169, 1919.

The Vitamin B_{12} Content of Certain Fishery Materials

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(*Received for publication May 7, 1952*)

ABSTRACT

Irregularities occurring in the microbiological assay of herring (*Clupea pallasi*) materials for vitamin B_{12} (cobalamin) are described and the probable reasons for the differences discussed. With the employment of a *Lactobacillus leichmannii* assay procedure and conditions under which vitamin B_{12} (cyano-cobalamin) and vitamin B_{12a} (hydroxo-cobalamin) caused identical growth response, it was found that normal commercial flame-dried herring meals had about the same total cobalamin content as specially prepared meals dried at 38° to 43.5°C. Chromatographic separation, elution and assay of the cobalamins in herring-meal samples indicated that substantially all of the activity found by direct assays of the meals was due to the vitamin itself. Hexane extraction had little effect on the cobalamin content, but heating the meals reduced it.

REVIEW OF PRELIMINARY EXPERIMENTS

During an investigation concerned with the nutritive value of herring meals (Tarr, Southcott and Bissett, 1950; Tarr, Bissett, Southcott, Biely and March, 1951) considerable difficulty was experienced in obtaining repeatable results in the assay for total cobalamins. Thus in one survey which extended over a period of several months 13 different herring materials including whole fish, press cake, press water, air-flow dried (ideal) meals, both before and after heating, and two commercial meals were assayed. Three different methods of extraction were used: (1) Aqueous suspensions were autoclaved 15 minutes at 120°C. at their natural pH (6.5–6.8) and filtered. (2) The materials were digested under toluene with 6.7 per cent of their weight of papain activated with cysteine (Tarr, Southcott and Ney, 1950) for two days at 37°C. and pH 7.0, autoclaved as in (1) and filtered. (3) The materials were digested with 6.7 per cent of Mylase P for two days at 37°C. under toluene and the digests treated as in (2). The filtrates were appropriately diluted and assayed with *L. leichmannii* 4797 as previously described using both sterilization in the medium and aseptic addition procedures (Tarr, 1951). In these initial assays vitamin B_{12a} was not available and the assay conditions may not have been such as to cause identical response of *L. leichmannii* to vitamin B_{12} and B_{12a} (see below).

About 175 separate determinations were made, individual materials being assayed from 11 to 29 different times. The results were extremely inconsistent and will only be referred to briefly. In general it appeared that higher apparent cobalamin recoveries resulted with the enzyme-digested materials than with aqueous autoclaved extracts, and that the aseptic addition of separately sterilized appropriately diluted extracts to the sterilized assay medium also occasioned higher assay values than did the sterilization of such extracts in the culture

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medium. However, in a few instances these findings were somewhat reversed. One extreme example of the irregular results obtained was with a commercial herring meal where a value of 1440 mμg. of cobalamin per g. was obtained using a Mylase P digested sample and the "aseptic addition" technique, and only 190 mμg. per g. when an autoclaved aqueous extract was sterilized in the culture medium. The various factors which are believed to be responsible for these irregularities have been reviewed elsewhere (Tarr, 1952). They probably include relative instability of cyano-cobalamin and much greater stability of hydroxo-cobalamin when incubated at 37°C. in the assay medium containing reducing agents under conditions where growth of *L. leichmannii* is rather delayed, formation of uncharacterized vitamin B_{12}-active substances in enzymic hydrolysates, and the greater susceptibility of hydroxo-cobalamin to destruction when sterilized in the particular assay medium in presence of reducing agents.

Several studies of the susceptibility of the vitamin B_{12}-active substances in chum salmon kidney and herring meal to alkali treatment (Stokstad, Jukes, Pierce, Page and Franklin, 1949) were carried out. It was found that over 90 per cent destruction invariably occurred following treatment of aqueous suspensions of the undigested materials. However, on alkali treatment of papain- and Mylase-hydrolysed kidney there remained a very substantial amount of apparent vitamin B_{12} activity, probably occasioned by the occurrence or formation of uncharacterized vitamin B_{12}-active substances such as those occurring in enzymic casein hydrolysates (Welch and Wilson, 1949). It is evident from this that differences in assay values for vitamin B_{12} as obtained before and after alkali treatment cannot always be relied upon to give an accurate picture of the true cobalamin content of a given material.

At this time, as a result of work reported elsewhere (Tarr, 1952) it became apparent that vitamin B_{12} occurs in natural protein materials largely as hydroxo-cobalamin, or as a similar form as judged by separation using partition chromatography (Woodruff and Foster, 1950). It was also found that cyano-cobalamin and hydroxo-cobalamin caused identical growth response of *L. leichmannii* in the medium of Skeggs, Nepple, Valentik, Huff and Wright (1950) when they were added aseptically to the medium under certain carefully regulated conditions. Moreover it was shown that cyanide treatment of crude herring-meal suspensions apparently occasioned only partial reversion of the hydroxo- to cyano-cobalamin, that this treatment did not affect the assay values obtained to any important extent and that usually at least 95 per cent of the apparent total cobalamins as found by direct assay were recovered following chromatographic separation, elution and assay. These findings have been verified and extended in the present work using the specific assay conditions referred to above.

PRESENT EXPERIMENTS

Table I records the results obtained in the assay of a number of herring preparations which were obtained or prepared in the same plant on the same day in November 1950, but not necessarily from completely identical fish. These

were subjected to several different digestion and extraction methods (Table I). The whole herring (a 1 in 5 aqueous suspension of blended material) and fish meal samples (1 in 10 aqueous suspensions) were digested for 48 hours under toluene with 5 per cent of papain (Nutritional Biochemicals Corporation) in the presence of 100 μg. per ml. of KCN at pH 7.0. In other instances digests or aqueous suspensions were extracted by autoclaving five minutes at 120°C. and pH 7.0 or 4.5 with or without 100 μg. per ml. of KCN (Table I).

TABLE I. Effect of papain digestion, extraction method and cyanide treatment on the cobalamin content of whole herring and various experimental and commercial herring meals, as determined by microbiological assay.

Material assayed	Mμg. of cobalamin per gram (dry weight)[a]				
	1	2	3	4	5
Whole herring	439	382	371 {303 / 379}	390	349 {367 / 409}
Ideal Nov. 1950 meal unheated	494	473	428	410	422
Ideal Nov. 1950 meal heated 60 min.	249	223	207	281	311
Ideal Nov. 1950 meal heated 120 min.	113	99	82	150	177
Ideal Nov. 1950 meal heated 180 min.	84	67	72	86	78
Hexane extracted ideal meal, unheated	406	416	401	383	368
Hexane extracted ideal meal, heated 60 min.	222	222	214	282	210
Hexane extracted ideal meal, heated 120 min.	106	103	122	117	107
Hexane extracted ideal meal, heated 180 min.	64	68	81	99	...
Commercial Feb. 1950 meal	409	354	379	354	369
Commercial Feb. 1951 meal	520	454	436 {418 / 400}	430	406 {451 / 414}

[a]The values in brackets are those obtained following chromatographic separation of the cobalamins, elution and assay. The numbers at the heads of the columns refer to the following preparative methods: (1) papain hydrolysed, autoclaved at pH 7.0 with 100 μg. per ml. of freshly added KCN; (2) papain hydrolysed, autoclaved at pH 4.5; (3) papain hydrolysed, autoclaved at 120° C. for 5 minutes at pH 7.0 after adding 100 μg. per ml. of KCN; (4) autoclaved at pH 7.0; (5) autoclaved at pH 7.0 with 100 μg. per ml. of KCN.

The results show that enzymic proteolysis, cyanide treatment and autoclaving at pH 4.5 or 7.0 had no important effect on the apparent cobalamin content of the various materials. The variations which did occur among samples may have been due to the inherent rather large error which often occurs in assays of this type (Brownlee and Lapedes, 1951). It is interesting to note that hexane extraction had no effect on the cobalamin content, that heating caused progressive destruction and that chromatographic separation, elution and assay indicated that the direct assay values were actually due to cobalamins and not to other vitamin B_{12}-active materials. It is of interest that, when calculated on a moisture- and fat-free basis, the average cobalamin content of the whole herring (100 fish sampled) was 460 mμg. per gram while that of the air-flow dried meal prepared from fish at about the same time, but not necessarily from identical material, averaged 480 mμg. per gram on the same basis. This indicates that much of the vitamin remains in the cooked flesh in the conventional cooking and pressing process. It will be noted that the two commercial meals compared favourably in their total cobalamin content with the air-flow dried product.

In a further experiment 10 per cent aqueous suspensions of two meals were digested with papain activated with cyanide under the conditions described above, or were merely heated. Samples of the digests were removed at intervals,

treated as recorded in Table II, and assayed for total cobalamins both directly and after chromatographic separation and elution of the vitamin. The results (Table II) show that papain digestion followed by autoclaving at 120°C. for five minutes does not liberate more vitamin B_{12} than does water extraction at 100 or 120°C., and that it may occasion fictitiously high recoveries as with the eight-hour hydrolysed ideal meal. It is probable that the occasional apparently high vitamin B_{12} content of certain fish meals recorded in former work (Tarr, Southcott and Ney, 1950; Tarr, Bissett, Southcott, Biely and March, 1951) may have been due to the formation of uncharacterized vitamin B_{12}-active substances in papain or Mylase P digests. In this previous work the fish-meal digests had usually been autoclaved in the assay medium using a reducing agent such as ascorbic acid, and casein digests are known to form vitamin B_{12}-active substances under such conditions (Welch and Wilson, 1949). In this, as in previous experiments (Tarr, 1952), the assay results obtained following chromatographic separation of the cobalamin were of similar order to those obtained by direct assay.

TABLE II. Effect of papain digestion on the cobalamin content of herring meals as determined by direct microbiological assay and assay following chromatographic separation of the vitamin.

Treatment of herring meal suspensions		Mμg. of cobalamin per gram dry weight			
		Commercial Nov. 1950 meal		Air-flow dried Feb. 1951 meal	
Papain digestion (hr.)	Heat treatment	Direct assay	Assay following chromatographic separation	Direct assay	Assay following chromatographic separation
No papain added	100° C. 30 min.	354	402 370	505	455
No papain added	120° C. 5 min.	354	343	436	462 488
0	120° C. 5 min.	348	353 401	416	505 548
4	120° C. 5 min.	366	412 410	440	525 550
8	120° C. 5 min.	447	366 362	1140	485 526
24	120° C. 5 min.	421	460	384	479
48	120° C. 5 min.	406	443	435	...
72	120° C. 5 min.	389	365	420	470

A further experiment was carried out by using a number of meals which had been prepared in the same plant from the same load of herring. In this experiment the ideal meal was prepared by drying the press cake in an air tunnel at 39° to 43°C. Samples of this meal were heated in a rotating drum (Harrison, 1950) for different lengths of time at 149°C., and a portion was extracted with hexane. The normal commercial meal was prepared by direct-flame drying of herring press cake (fire-box temperature 538°–620°C., stack temperature 108°–125°C. and meal discharged at 46°–54°C.); and the overheated commercial meal by similar drying at a higher temperature (fire-box temperature 650°–928°C., stack temperature 158°–171°C. and meal discharged 79°–93°C.).

The results (Table III) confirm those obtained in the first experiment regarding the similar cobalamin content of the normal commercial meal and the ideal meal, and also those which showed that overheating the ideal and commercial meals caused destruction of the cobalamin but that hexane extraction had little effect.

TABLE III. The cobalamin content of certain experimental and commercial herring meals as determined using extracts prepared by heating aqueous suspensions at pH 6.8 and 4.5 for 5 minutes at 120° C.

Material assayed	Mμg. of cobalamin per g. dry weight	
	pH 6.8	pH 4.5
Ideal meal untreated	396	394
Ideal meal heated 20 min. at 149° C.	355	335
Ideal meal heated 40 min. at 149° C.	282	329
Ideal meal heated 60 min. at 149° C.	254	302
Ideal meal heated 80 min. at 149° C.	237	248
Ideal meal heated 120 min. at 149° C.	202	227
Ideal meal hexane extracted, unheated	394	...
Commercial herring meal, normal drying	349	...
Commercial herring meal, purposely overheated	240	...

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REFERENCES

BROWNLEE, K. A., and D. N. LAPEDES. *J. Bact.*, **62**, 433–434, 1951.

HARRISON, J. S. M. *Fish. Res. Bd. Canada, Prog. Rept. Pac.*, No. 85, 86–87, 1950.

SKEGGS, H. R., H. M. NEPPLE, K. A. VALENTIK, J. W. HUFF and L. D. WRIGHT. *J. Biol. Chem.*, **184**, 211–221, 1950.

STOKSTAD, E. L. R., T. H. JUKES, J. PIERCE, A. C. PAGE and A. L. FRANKLIN. *J. Biol. Chem.*, **180**, 647–654, 1949.

TARR, H. L. A. *Can. J. Technology*, **29**, 391–400, 1951.
Can. J. Technology, **30**, 265–279, 1952.

TARR, H. L. A., H. M. BISSETT, B. A. SOUTHCOTT, J. BIELY and B. E. MARCH. *Fish. Res. Bd. Canada, Prog. Rept. Pac.*, No 87, 42–46, 1951.

TARR, H. L. A., B. A. SOUTHCOTT and H. M. BISSETT. *Fish. Res. Bd. Canada, Prog. Rept. Pac.*, No. 85, 83–85, 1950.

TARR, H. L. A., B. A. SOUTHCOTT and P. W. NEY. *Food Technology*, **4**, 354–357, 1950.

WELCH, A. D., and M. F. WILSON. *Arch. Biochem.*, **22**, 486–489, 1949.

WOODRUFF, H. B., and J. C. FOSTER. *J. Biol. Chem.*, **183**, 569–576, 1950.

The Action of Hydroxylamine on Bacteria

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ABSTRACT

When hydroxylamine was incorporated into muscle of halibut, lingcod and coho salmon in concentrations varying from 24 to 240 μg. per gm. (24 to 240 parts per million) it caused a much more marked delay in the rate of bacterial spoilage of samples stored at 0°C., and in one instance at 25°C., than did 200 μg. per gm. of sodium nitrite. From 5 to 50 μg. of hydroxylamine per ml. of nutrient broth either partially or entirely inhibited the growth of ten species of fish spoilage bacteria at 25°C. and pH 5.9 to 7.5. Somewhat similar results were obtained with a culture of a food poisoning strain of *Staphylococcus aureus* and the strict anaerobe *Clostridium botulinum*, except that these organisms were in general less sensitive and that most effective inhibition was obtained at pH 5.9.

A brief report concerning this investigation, which included a short literature survey, was published some time ago (Tarr, 1945). Since that time there have been additional contributions to the literature regarding the bacteriostatic action of hydroxylamine (Fedorov, 1951; Hietaranta, 1949), and because further interest has been taken in its ability to preserve high protein foods (Lovtrup, 1949) it was thought expedient to record in detail the results of the work completed at this Station.

EXPERIMENTAL

Inhibition of Growth of the Mixed Bacterial Flora of Fish Muscle

In these experiments fillets of the fish studied were blended in a Waring blendor, and 5 ml. of either water or a freshly prepared solution of chemically pure hydroxylamine (as the calculated amount of the hydrochloride) or sodium nitrite were incorporated into 45 g. of the muscle. The treated samples were stored in glass-covered sterilized 250 ml. beakers, direct bacterial counts being made at stated intervals (Tarr, 1943). The results of these experiments are given in Tables I, II and III.

Table I. Effect of hydroxylamine on keeping quality of lingcod flesh stored at 0° C. (Initial pH of flesh 6.6 and bacterial count 0.5 × 10^6 per gram.)

NH_2OH (μg. per g.)	Bacteria × 10^6 per g. after 5 days	Bacteria × 10^6 per g. after 9 days
0	650	Putrid
5	480	Putrid
10	120	Putrid
25	70	Very stale
50	22	850 (stale)

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When taken collectively they show that hydroxylamine is in general a much more effective agent for counteracting bacterial spoilage of fish flesh than is sodium nitrite. It is true that results with coho salmon indicate no important difference in the bacteriostatic effect of the two substances at 200 μg. per g. level. This is probably a rather exceptional instance, for the initial muscle pH was low, and it is known that salmon flesh does not become increasingly alkaline during bacterial spoilage as readily as does that of many other species of fish. With herring (Table II) the initial pH of the muscle was much higher, and hydroxylamine was more effective than sodium nitrite when used in about one-ninth the concentration. It was shown some time ago that the effectiveness of nitrite as a bacteriostat for fish flesh depends directly on the acidity of the tissue (Tarr and Sunderland, 1940), but with hydroxylamine, experiments using pure cultures of bacteria indicate that this is not the case (see below).

TABLE II. Effect of hydroxylamine and sodium nitrite on keeping quality of herring flesh stored at 0° C. and 25° C. (Initial pH 6.7 and bacterial count 0.85 × 10^6 per gram.)

Concentration of inhibitor (μg. per g.)	Storage temperature	Bacteria × 10^6 per g. after 1 day	4 days	9 days	15 days
0 NH_2OH	0° C.		164	*a*	
12 NH_2OH	0° C.		58	*a*	
24 NH_2OH	0° C.		6.6	*a*	
60 NH_2OH	0° C.		2.7	1300	*a*
120 NH_2OH	0° C.		0.10	84	*a*
240 NH_2OH	0° C.		0.08	52	220
200 $NaNO_2$	0° C.		91	*a*	
0 NH_2OH	25° C.	370			
60 NH_2OH	25° C.	138			
240 NH_2OH	25° C.	3.8			
200 $NaNO_2$	25° C.	290			

a Samples putrid and discarded.

TABLE III. Effect of hydroxylamine and sodium nitrite on keeping quality of halibut and coho salmon flesh stored at 0° C. (Initial pH of flesh 6.35 and 6.2 respectively and bacterial counts < 0.013 × 10^6 per gram.)

Days stored	Bacteria × 10^6 per g. Halibut			Coho salmon		
	Untreated	$NaNO_2$ (200 μg. per g.)	NH_2OH (200 μg. per g.)	Untreated	$NaNO_2$ (200 μg. per g.)	NH_2OH (200 μg. per g.)
4	14	0.52	0.026	0.15	< 0.013	< 0.013
8	2700	0.77	0.093	300	< 0.013	< 0.013
12	*a*	28.0	5.0	4100	0.079	0.04
18	*a*	31000	4100	*a*	22	35

a Samples putrid and discarded.

TABLE IV. Effect of different hydroxylamine concentrations on growth of fish spoilage bacteria in nutrient broth at various pH values. Initial inocula are given in brackets. (− = No growth; S+ = slight growth; + = normal growth.)

Initial pH	NH_2OH μg. per g.	Growth after days with[a]															
		Culture 1(17.0) (*Micrococcus* sp.)			Culture 2(3.0) (*Micrococcus* sp.)			Culture 6(6.8) (*Flavobacterium* sp.)			Culture 7(0.25) (*Micrococcus* sp.)			Culture 9(3.8) (*Achromobacterium* sp.)		
		1	2	28	1	2	28	1	2	28	1	2	28	1	2	28
5.9	0	−	−	−	+	+	+	+	+	+	+	+	+	+	+	+
	5	−	−	−	−	+	+	S+	+	+	S+	+	+	S+	+	+
	10	−	−	−	−	−	+	−	−	−	−	S+	+	−	+	+
	25	−	−	−	−	−	−	−	−	−	−	−	−	−	+	+
	50	−	−	−	−	−	−	−	−	−	−	−	−	−	+	+
6.3	0	−	−	+	+	+	+	+	+	+	+	+	+	+	+	+
	5	−	−	+	+	+	+	−	+	+	+	+	+	−	+	+
	10	−	−	−	−	−	+	−	−	−	S+	+	+	−	−	+
	25	−	−	−	−	−	−	−	−	−	−	−	+	−	−	+
	50	−	−	−	−	−	−	−	−	−	−	−	−	−	−	+
7.1	0	−	−	+	+	+	+	+	+	+	+	+	+	+	+	+
	5	−	−	+	+	+	+	+	+	+	+	+	+	S+	+	+
	10	−	−	−	−	+	+	−	−	−	+	+	+	−	−	+
	25	−	−	−	−	+	+	−	−	−	−	−	+	−	−	−
	50	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
7.5	0	−	−	+	+	+	+	+	+	+	+	+	+	+	+	+
	5	−	−	−	+	+	+	S+	+	+	+	+	+	−	+	+
	10	−	−	−	S+	+	+	−	−	−	S+	+	+	−	−	−
	25	−	−	−	−	−	+	−	−	−	−	−	+	−	−	−
	50	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−

[a] In only 4 instances was growth recorded after 28 days when none was observed after 14 days.

TABLE IV (*continued*).

Initial pH	NH_2OH μg. per g.	Growth after days with[a]															
		Culture 11(1.9) (*Micrococcus* sp.)			Culture 14(0.88) yeast			Culture 15(1.9) yeast			Culture 16(3.8) (*Achromobacterium* sp.)			Culture 22(4.0) (*Achromobacterium* sp.)		
		1	2	28	1	2	28	1	2	28	1	2	28	1	2	28
	0	−	+	+	+	+	+	+	+	+	+	+	+	+	+	+
	5	−	S+	+	+	+	+	+	+	+	+	+	+	+	+	+
5.9	10	−	S+	+	S+	S+	+	−	+	+	−	S+	+	+	+	+
	25	−	−	−	−	−	+	−	−	−	−	−	−	−	S+	+
	50	−	−	−	−	−	−	−	−	−	−	−	−	−	−	+
	0	+	+	+	−	+	+	+	+	+	+	+	+	+	+	+
	5	+	+	+	−	+	+	S+	+	+	+	+	+	+	+	+
6.3	10	+	+	+	−	−	+	−	+	+	+	+	+	+	+	+
	25	−	−	+	−	−	+	−	−	+	−	−	−	−	−	+
	50	−	−	−	−	−	−	−	−	−	−	−	−	−	−	+
	0	+	+	+	−	+	+	+	+	+	+	+	+	+	+	+
	5	S+	+	+	−	+	+	S+	+	+	+	+	+	+	+	+
7.1	10	−	S+	+	−	−	+	−	+	+	+	+	+	+	+	+
	25	−	−	+	−	−	+	−	−	+	−	−	−	−	−	+
	50	−	−	−	−	−	−	−	−	−	−	−	−	−	−	+
	0	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
	5	S+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
7.5	10	−	−	+	S+	+	+	S+	+	+	S+	+	+	S+	+	+
	25	−	−	+	−	−	+	−	−	+	−	−	−	−	S+	+
	50	−	−	+	−	−	−	−	−	−	−	−	−	−	−	+

[a] In only 4 instances was growth recorded after 28 days when none was observed after 14 days.

Inhibition of Growth of Fish Spoilage and Certain Food-Poisoning Organisms at Different pH Values

The ten cultures used in the first experiment were those isolated in previous work (Tarr, 1939). They had been transferred at irregular intervals on Bacto nutrient agar and stored at about 0° to 3°C. The organisms were grown for two days at 25°C. on this medium (pH 7.0), the growth was suspended in sterile 0.1 per cent NaCl solution and the cells were concentrated by centrifuging. The cells thus prepared were stored at 0°C. and used within 24 hours of preparation. Light suspensions were prepared in sterile saline, the number of organisms present being determined with a Petroff-Hauser counting chamber. The size of inoculum used with each organism is given in Table IV. The bacteriostatic effect of hydroxylamine was investigated as follows. Bacto nutrient broth (double strength) 1.5 ml., and 0.5 ml. of 0.2 M. phosphate buffer of different pH values were added to 13 × 150 mm. tubes and sterilized at 120°F. for 15 minutes. Immediately before use 0.5 ml. of Seitz filter-sterilized hydroxylamine hydrochloride solution of appropriate concentration was added to each tube followed by 0.5 ml. of the saline suspension of the culture investigated. The tubes were incubated at 25°C.

The results of the experiment (Table IV) show that there was no obvious correlation between the pH of the medium and the bacteriostatic or bactericidal action of hydroxylamine as was found with sodium nitrite using identical cultures

Table V. Effect of different hydroxylamine concentrations on growth of *Clostridium botulinum* and *Staphylococcus aureus* at various pH levels. (− No visible growth after 2 days' incubation.)

NH_2OH (μg. per g.)	Growth after days							
	C. botulinum				*S. aureus*			
	Initial pH	7	14	28	Initial pH	1	2	14
0		+	+	+		+	+	+
5		−	+	+		+	+	+
10	5.9	−	+	+	5.9	+	+	+
25		−	−	−		−	−	−
50		−	−	−		−	−	−
0		+	+	+		+	+	+
5		+	+	+		+	+	+
10	6.6	+	+	+	6.3	+	+	+
25		+	+	+		−	−	+
50		−	−	−		−	−	−
0		+	+	+		+	+	+
5		+	+	+		+	+	+
10	6.9	+	+	+	7.1	+	+	+
25		+	+	+		−	+	+
50		−	−	−		−	−	+
0		+	+	+		+	+	+
5		+	+	+		+	+	+
10	7.65	+	+	+	7.5	+	+	+
25		+	+	+		+	+	+
50		−	−	+		−	−	+

(Tarr, 1941). Hydroxylamine in 5 μg. per ml. concentration only exhibited a mild and transient bacteriostatic action in about one-third of the instances in which it was used. In 10 μg. per ml. concentration it was generally more effective but not consistent in its action. Thus with culture 6 at all four pH values studied no growth occurred at this concentration, while with culture 22 the identical concentration was practically without effect. The higher concentrations investigated either severely inhibited or entirely prevented growth.

In a further experiment the effect of hydroxylamine on growth of *Staphylococcus aureus* (food-poisoning strain) and *Clostridium botulinum* (Type A) at 37°C. was investigated. The conditions used for *S. aureus* were the same as those in the experiment with the fish-spoilage organisms. *C. botulinum* was cultured 48 hours in fish-digest broth (Tarr, 1942) and this medium was used in half the normal concentration in the hydroxylamine experiments.

The results of this experiment (Table V) show that these organisms were, in general, less sensitive to hydroxylamine than the fish-spoilage organisms. With *S. aureus* hydroxylamine was slightly more toxic at the lower pH values. With *C. botulinum* 5 and 10 μg. per ml. caused bacteriostasis and 25 μg. per ml. complete inhibition of growth at pH 5.9, but this effect was not noticed at higher pH values where 50 μg. per ml. was the only effective concentration.

DISCUSSION

Several years ago experiments with cats and white rats which were conducted at this Station indicated that sodium nitrite, when ingested in amounts which were ten times greater than the maximum which would be ingested in a corresponding ration of cured meats containing the permitted level of 200 parts per million, had no important effect on their growth rate nor on the size of their liver, kidneys, spleen, heart, thyroid, adrenals or lungs (Tarr and Carter, 1942). Subsequently the Canadian Food and Drug Regulations (1949, page 76) were altered to permit the inclusion of sodium nitrite in fresh fish as follows: "Class I preservatives shall be . . . *and in cured meats and fresh fish only* sodium nitrite of which the proportion shall not exceed 200 parts per million of a finished food". Recently Riemann (1950) has reviewed the earlier work of Jacobsen, Munk and Milwertz (1939) on toxicity of hydroxylamine and has carried out experiments to determine its oral toxicity for white rats. In his experiments the level of hydroxylamine fed was almost double that of sodium nitrite as fed to white rats by Tarr and Carter (1942). His results showed that the animals which were fed these rather high concentrations of hydroxylamine became slightly heavier than the controls, that they developed greatly enlarged spleens and that the thyroid glands were markedly reduced in size.

The present results have emphasized that hydroxylamine is effective in retarding bacterial spoilage of fish when used in much lower concentrations than sodium nitrite, and that the effectiveness of the former is largely independent of the pH of the flesh while that of the latter is pronounced only when the flesh pH is low. It is conceivable then that hydroxylamine might be a much more effective bacteriostat for fish flesh than sodium nitrite, but at present the information re-

garding its toxicity would appear to be too limited to permit of its inclusion in foods intended for human use.

ACKNOWLEDGMENT

I am indebted to Dr. L. A. Ranta of the University of British Columbia for providing the *Clostridium botulinum* and *Staphylococcus aureus* (Wood strain) cultures used in this work.

REFERENCES

FEDOROV, M. V. *Doklady Akad. Nauk U.S.S.R.*, **50**, 501–504, 1951.
HIETARANTA, M. *Svenska Majeritidn.*, **41**, 63–65 and 75–78, 1949.
JACOBSEN, E., C. MUNK and I. MILWERTZ. *Nord. Med.*, **3**, 2851–2858, 1939.
LOVTRUP, E. Cited by H. Riemann, *Act. Pharmacol.*, **6**, 285–292, 1950.
RIEMANN, H. *Act. Pharmacol.*, **6**, 285–292, 1950.
TARR, H. L. A. *J. Fish. Res. Bd. Canada*, **4**, 367–377, 1939.
Ibid., **5**, 265–275, 1941.
Ibid., **6**, 74–89, 1942.
Ibid., **6**, 119–128, 1943.
Ibid., **6**, 349–350, 1945.
TARR, H. L. A., and N. M. CARTER. *J. Fish. Res. Bd. Canada*, **6**, 63–73, 1942.
TARR, H. L. A., and P. A. SUNDERLAND. *J. Fish. Res. Bd. Canada*, **5**, 148–163, 1940.

The Central Nervous System of Barnacles (Cirripedia)

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INTRODUCTION

The nerves of barnacles present many points of interest. For one thing, they show in one sub-class of animals a gradual concentration of the nerve system, from the primitive to the most highly developed. They also show some links with the less specialized Crustacea from which they were derived.

Very little is known about the origin of barnacles, but they must have been well established and have found the place in nature to which they were best adapted before they became well enough distributed to leave fossils.

There is a certain resemblance in the arrangements of the nerves of all barnacles, but the arrangement of those of the Lepadidae, or pedunculate barnacles (Figures 1–3) show that they are more primitive than the Balinidae or sessile barnacles (Figures 4–7). This agrees with what is known of the palaeontology of the group. According to Dr. T. H. Withers of the British Museum (1928), the first crustacean remains were found in Palaeozoic formations, while the first barnacle remains, those of a pedunculate barnacle, were found in the Triassic formation. But the remains of the more advanced sessile barnacles have not been found earlier than the Cretaceous.

All the drawings in this paper were made by camera lucida from original dissections and they are of course highly magnified.

In describing the nerve systems of barnacles, the names designating the parts are those used by Darwin in his Monographs (1851, 1854). The parts listed below are to be found in all the systems figured; they are arranged according to the symbols by which they are designated in Figures 1–7.

1. The infra-oesophageal ganglion, which lies back of the oesophagus or throat.
2. The supra-oesophageal ganglion, which is in front of the oesophagus. Several very small nerves are connected to the commissure that connects the two ganglia that form the supra-oesophageal ganglion, and they can be traced to the gnathites, or mouth parts. These small nerves are shown only in Figure 7 but are to be found in all barnacles.
3. The circum-oesophageal chord, formed by the two nerves that connect the supra-oesophageal and the infra-oesophageal ganglia.
4. Nerves leading to the scutum adductor muscle, the large muscle that closes the coverplates.
6. Nerves leading to the network of nerves that are near the surface of the body. They correspond with the antennular nerves of crustaceans.
7. The optic nerves, leading to the eyes.

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9. The splanchnic nerves, running deeply into the body and supplying the viscera. A branch from these nerves runs to the ovarian tubes.

I–VI. Nerves leading to the cirri. They divide on entering the pedicel of each cirrus, one branch going to each ramus of the cirrus, and another branch supplying its base.

PP. Nerves leading to the penis. In some species these are in the same sheaths as the nerves of the sixth cirri, and branch from it. In others they are connected directly to the infra-oesophageal ganglion.

OE. This marks the position of the oesophagus.

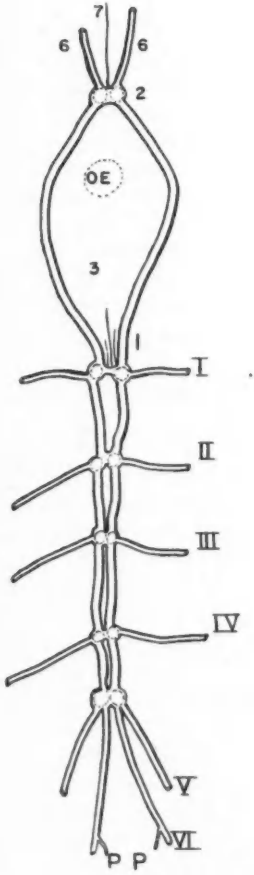

FIGURE 1. Nerve system of *Lepas hilli* Leach. (For explanation of symbols see pp. 76–77.)

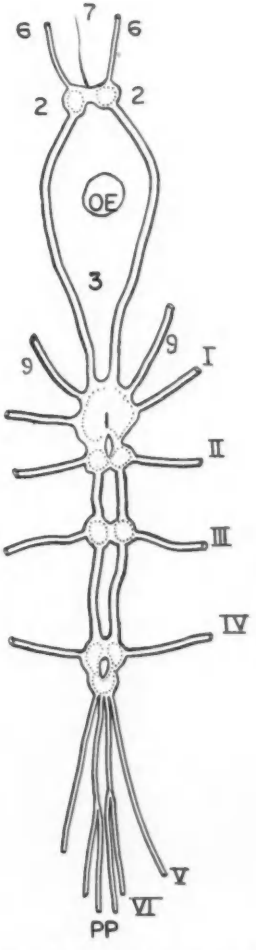

FIGURE 2. Nerve system of *Mitella polymerus* (Sowerby). (For explanation of symbols see pp. 76–77.)

The position in which a barnacle lies should be clearly understood. It is fastened to its shell on its back, and all the figures show a ventral view. The cirri, or legs, are toward the observer.

DESCRIPTIONS

The Lepadidae have the ladder-like arrangement of the nerves resembling that of a primitive crustacean. But even in this group there is an advance in concentration. *Lepas hilli* Leach (Figure 1) is the most primitive as there is very little concentration except the shortening of the commissures between the ganglia of the nerves leading to the cirri. These commissures are long and double in the primitive crustacea, but are short and single in the barnacles. *Mitella polymerus* (Sowerby) (Figure 2) shows more concentration as the number of main ganglia has been reduced. The ganglia of the fourth, fifth and sixth cirri are close together and are partly coalesced with the ganglion of the penis. In the infra-oesophageal ganglion the ganglia of the nerves leading to the second cirri are close together and are almost coalesced with the ganglia of the first cirri, the ganglia of the splanchnic and the ganglia of the circa-oesophageal chord.

The nerves of *Smilium spinosum* Annondale (Figure 3) show another step in concentration. In it the ganglia of the nerves leading to the fourth, fifth and sixth cirri have drawn together and coalesced with that of the penis forming a dumb-bell shaped ganglion. The ganglia of the third cirri have coalesced, as have those of the second cirri. The ganglia of the first cirri, the splanchnic and the circum-oesophageal chord are completely coalesced. The two ganglia that form the supra-oesophageal ganglion are also coalesced, and this is the only barnacle that I have seen with these ganglia coalesced. The optic nerve and those nerves that are connected to the anterior end of the ganglion are all enclosed in three sheaths that are close together. These nerves are 6 and 7 in the figure.

The Balanidae have a more concentrated nerve system than the Lepadidae as they have only two main ganglia, the supra-oesophageal and the infra-oesophageal. The supra-oesophageal is made up of two ganglia that are connected by a commissure. The infra-oesophageal is made up of several that have been completely coalesced. There are 16 main nerves connected to this ganglion in some species, in others there are 18. There are also several small nerves connected to the dorsal side that are not shown in the figures. It is not always possible to show the exact shape of the infra-oesophageal ganglion as even the weight of the cover-glass may distort it.

The arrangement of the nerves in *Balanus nubilus* Darwin (Figure 4) is typical of the arrangement found in many of the Balanidae, but there are variations in every species. In *B. nubilus* the large nerve (No. 4 in the figure) leading to the scutum adductor muscle does not run direct to the muscle but joins with the splanchnic nerve, then it branches from it. This is shown in Figure 4 at 10 in the broken circle. Also the nerves to the penis are connected direct to the ganglion and are not in the same sheath as the nerves of the sixth cirri, as in so many other species.

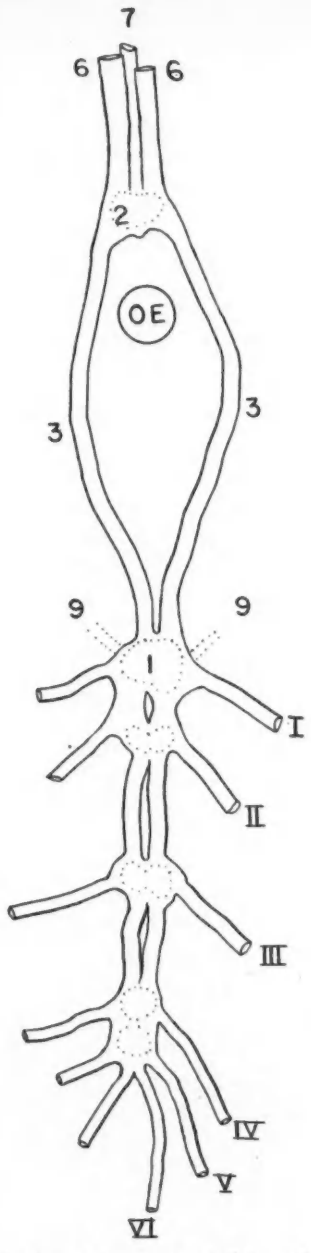

FIGURE 3. Nerve system of *Smilium spinosum* Annondale. (For explanation of symbols see pp. 76–77.)

There are two species of *Chthamalus* Ranzani found on the North American Pacific coast, *Chthamalus dalli* Pilsbry (Figure 5) and *C. microtretus* Cornwall. Both of these species are found only in the intertidal zone. They both have very great development of the infra-oesophageal ganglion, it being half as long as the oesophageal chord. The arrangement of the nerves is much the same as that of *Balanus nubilus,* but there is a difference in the connection of the nerve connected with the scutum adductor muscle, they do not join the splanchnic, but a branch from the splanchnic joins it. This connection is shown in Figure 5 in the broken circle, and is found in many intertidal species.

The arrangement of the nerves in *Balanus altissimus* (Figure 7) shows variations worthy of mention. The nerves to the penis and the nerves to cirri V and VI all lie in the same sheath for some distance. The nerves leading to the

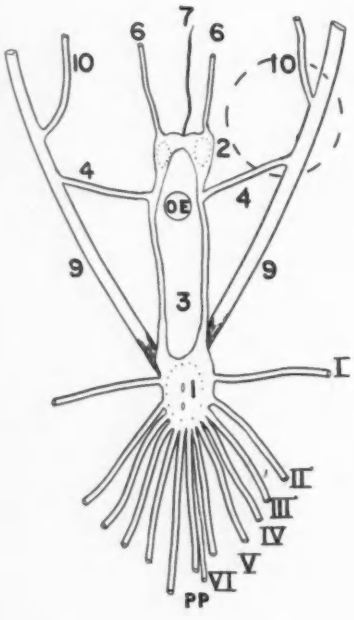

FIGURE 4. Nervous system of *Balanus nubilus* Darwin. 10, branch from splanchnic nerve to scutum adductor muscle. (For other symbols see pp. 76–77.)

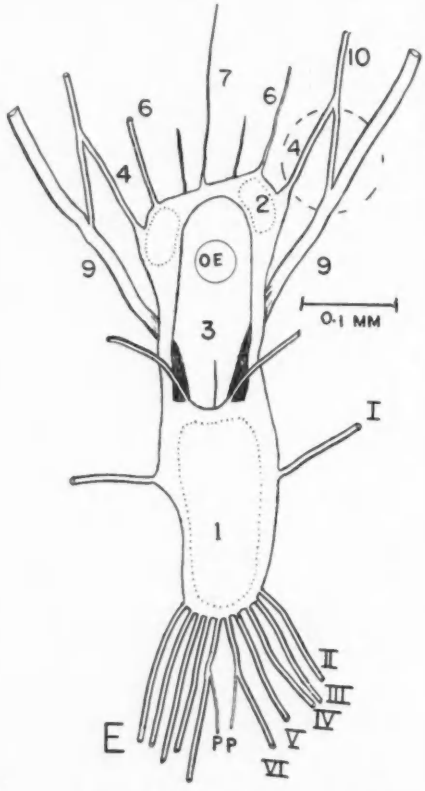

FIGURE 5. Nerve system of *Chthamalus dalli* Pilsbry. 4, branch from circum-oesophageal chord to scutum adductor muscle. 10, branch from splanchnic nerve to scutum adductor muscle. (For other symbols see pp. 76–77.)

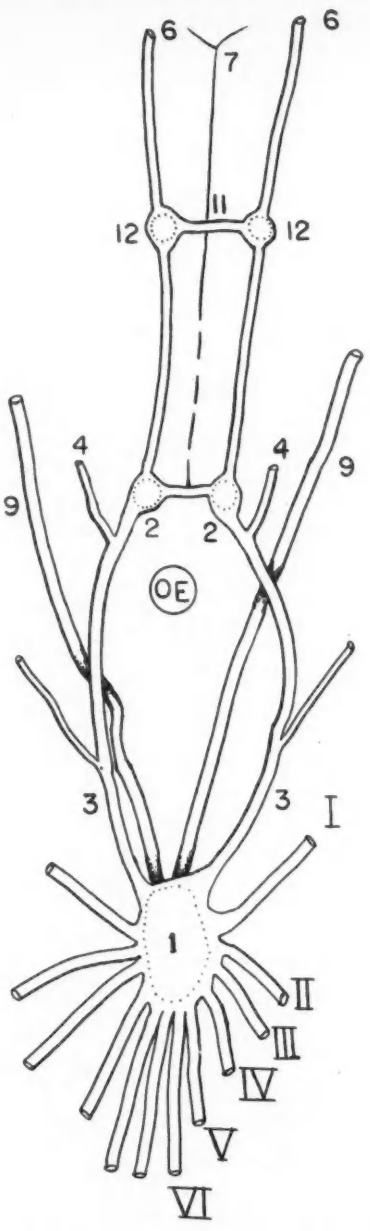

FIGURE 6. Nerve system of *Coronula complanata* (Mörch). 4, branch from circumoesophageal chord to scutum adductor muscle. 11, commissure between ganglia (12). (For other symbols see pp. 76–77.)

scutum adductor muscle run direct from the oesophageal chord to the muscle. They are joined by branches from the splanchnic nerve, as shown in the broken circle at Number 5 in Figure 7. This is the same arrangement as found in *Chthamalus dalli*. Another peculiarity about this species is the way the nerves connected to the eyes are arranged. Usually there is a single sheath that is connected to the commissure joining the ganglia that make the supra-oesophageal ganglion, and the optic nerves lie in this sheath. In this species there is a separate nerve to each eye (No. 7, in Figure 7). I have not found any other barnacle

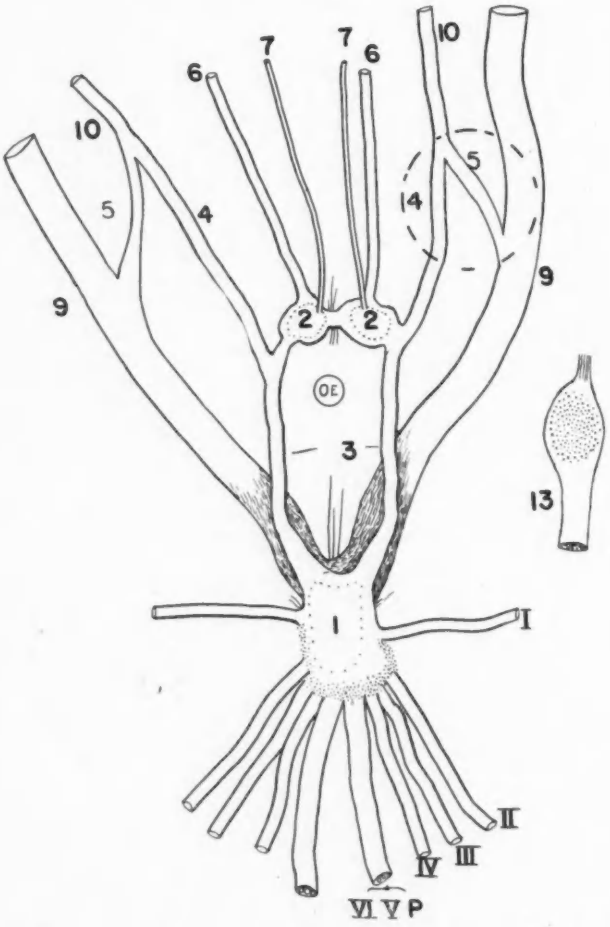

FIGURE 7. Central nervous system of *Balanus altissimus* Cornwall. 1, infra-oesophageal ganglion—its exact shape cannot be distinguished. 4, branch from circum-oesophageal chord to scutum adductor muscle. 10, nerve leading to scutum adductor muscle. 13, one of the eyes, much enlarged. (For other symbols see pp. 76–77.)

with a separate nerve leading to each eye. One of the eyes is shown, highly magnified, at 13 in the figure. It appears to be a ganglion that is coated with small pellets of a dark colour. They are not united in a continuous layer, and no cornea can be seen. The eyes are situated close under the translucent covering of the prosoma in such a position that they will be exposed to light when the coverplates are opened.

The whale barnacle *Coronula complanata* (Mörch) (Figure 6) has a peculiar arrangement of the nerves as there is a cross commissure in front of the supra-oesophageal ganglion. This is present on at least one of the primitive Crustacea, but according to Lankester (1909) its presence "is extremely doubtful". Yet in Lankester's Treatise on Zoology (1909) there is a drawing of the nerve system of *Branchinecta paludosa* (O. F. Müller) by Professor Sars, and it shows a cross commissure in front of the supra-oesophageal ganglion. The cross commissure in *C. complanata* is Number 11 in Figure 6, and the ganglia at each end of it are the same size as the ganglia that make the supra-oesophageal ganglion (No. 12 in Figure 6). This commissure connects the two nerves (No. 6) that supply the network of nerves that are near the surface of the body. The supra-oesophageal ganglion, the cross commissure and the nerves (6) are enclosed in a nearly transparent sheath.

In Professor Gruvel's Monograph on the Cirripedia (1905) he shows the infra-oesophageal ganglion with well-marked grooves that make it appear as if it were made of several ganglia that are not completely coalesced. *Balanus tintinnabulum* (Linnaeus) and *Coronula diadema* (Linnaeus) are shown in Plates 396 and 397 in this way. In Darwin's Monograph (1854), there are drawings of both these species (Plate XXVII), and he does not show any indication of this ganglion having been made of several that have not been completely coalesced. In dissecting both these species, and many others of the *Balinidae*, I have seen no grooves or any external mark to indicate that it is made up of several. In some species there is a row of shallow depressions as shown in the figure of *Balanus nubilus* (Figure 4).

As stated above, there is a difference in the arrangement of the nerves of the barnacles from deep water and those from the intertidal zone. There is also a difference in their action when taken from the water. Those from below low-tide mark will keep on opening their coverplates and going through the eating motion till they dry. Those from the intertidal zone will close their coverplates and keep them closed till again covered by water.

NOTE ON METHOD OF DISSECTING

A great deal of difficulty was encountered when I first started to search for the nerves of barnacles. As have many others, I found it very difficult to distinguish the nerves from other tissues. Dr. W. K. Fisher of the Hopkins Marine Station suggested a method that has proved very effective. All calcareous matter must be removed from the specimen to be examined, then it is placed in a ten per cent solution of nitric acid. This will soften *all* the fibres, but the nerve fibre is softened more slowly than is the muscle. The specimen must be removed from

the solution and washed just before the nerve fibres are attacked by the acid. This time can only be found by experiment. I have left large specimens in the solution for an hour, but it is very easy to spoil the specimen by being in the acid too long.

At first it is difficult to find the nerves, and the method that I have used may be found helpful. Cement a piece of cork in a small receptacle, pin the specimen to the cork with the mouth up. If the mouth is removed, the oesophageal chord, or collar ring, will be found under it.

LITERATURE CITED

DARWIN, CHARLES. 1851. A Monograph on the Sub-Class Cirripedia. The *Lepadidae*, or pedunculated barnacles. (See pp. 46–49.)

1854. A Monograph on the Sub-Class Cirripedia. The *Balanidea*, or sessile barnacles. (See pp. 103–112, and Plate XXVII.)

GRUVEL, A. 1905. Monograph des cirrhipedes ou thécostracés. Libraires de l'Academie de Médecine. (See pp. 402–410, Plates 397, 398.)

LANKESTER, E. RAY. 1909. A Treatise on Zoology. Part VII. (See Fig. 11.)

WITHERS, T. H. 1928. Catalogue of the fossil Cirripedia in the Department of Geology, British Museum (Natural History). Vol. I, Triassic and Jurassic. (See pp. 21, 24, 44.)

Micronereis nanaimoensis sp. n. : with some Notes on its Life-History

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(*Received for publication August 5, 1952*)

ABSTRACT

Micronereis nanaimoensis sp. n. is described from the region of Nanaimo, British Columbia. The definition of *Micronereis* is slightly emended to include the species. It differs from its only congener, *M. variegata* Claparède of Europe, in being much larger, in possessing palps, in having more varied setae and different specialized crotchets in the males, and in colour. Swarming, pairing and egg-laying occur from late March to the end of May. The eggs are moulded by the female into masses covered by jelly, which are deposited on leaves of *Zostera, Ulva,* or other seaweeds, near low-tide mark, and are accompanied by her in the jelly until the larvae become free-swimming. In the laboratory, larvae left the egg-mass after 10–16 days, in cold water. Larval development up to the three-segmented stage is illustrated, and supports the inclusion of *Micronereis* in Nereidae.

INTRODUCTION

The polychaete which is the subject of this paper has interested us for many years, but only recently have we had opportunity to obtain any detailed information on its morphology and life-history. The first mention of its occurrence in Departure Bay is in a paper by L. N. Ramsay (1914) who examined four specimens found "inhabiting globular masses of mucus on brown *Ulva*" dredged there by F. A. Potts in 1911. Ramsay compared these specimens with examples of *Micronereis variegata* Claparède collected on the south coast of England and with descriptions of that species given by Claparède (1863) and by McIntosh (1910) and concluded that they were identical. Ten years later it was listed by one of us (E. Berkeley, 1924) as of common occurrence throughout the Nanaimo region and more recently (1948) we have repeated the record. In both the latter cases, although the name *Micronereis variegata* is used, reference is made to points of divergence from the descriptions of that species and, in the second, it is suggested that reclassification may be necessary. This we now find to be the case.

There does not appear to be any record of the occurrence of any species of *Micronereis,* excepting these from the Nanaimo region, outside Europe. In Europe *Micronereis variegata* has been reported from both sides of the English Channel and from the Atlantic and Mediterranean coasts of France, found littorally amongst algae and *Lithoamnion,* and it has, hitherto, been the only representative of its genus.

The distribution of the species dealt with in this paper may not be so limited as it would appear to be, since the period during which its collection has been found possible is very short and its detection presents some difficulties. Our own first specimens were collected in the same way as were those recorded by

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Ramsay, from pieces of drifting algae taken in the dredge or floating inshore during the spring or very early summer months. They were invariably surrounded by a more or less spherical mass of jelly-like substance inside which they were accompanied by a mass of eggs at various stages of development. Later these jelly-masses were found at or near low tide mark attached to *Zostera*, *Ulva*, or other algae, during the early spring low tides at a number of points around the coast in the neighbourhood of Departure Bay and, rarely, free-living individuals were found in like situations. The jelly-masses are usually coated with diatoms and detritus making their detection difficult even when the plants carrying them are partially submerged and floating, and a good light and still water are essential: it is more difficult to find them after the tide has receded and the plants are lying on the beach. An additional difficulty is that a number of other organisms spawn on the same beds at the same time as *Micronereis* and, unless visibility conditions are very good, confusion with other spawn masses is easy. On the other hand one of the other spawning organisms, the pelagic nudibranch *Melibe leonina* (Gould), serves as a useful indicator of the probable presence of *Micronereis*. This animal is very conspicuous when spawning on the *Zostera* beds and, when it is seen, a diligent search will almost always disclose at least a few of the jellies. They are not usually to be found in the same localities after the end of May and we have not yet succeeded in obtaining larval forms either by towing over the beds or by washing the sand of which they are composed.

Early in 1952 we witnessed the swarming of the species over the *Zostera* and *Ulva* beds for the first time and were able to collect free-swimming mature specimens of both sexes. With this material we have been able to make some observations on its pairing, egg-laying, and early larval development. Our data on its life-history are yet far from complete, but, considering its apparent scarcity and the many characteristics in which it differs from the family it represents, such information as we have seems worth recording.

TAXONOMY

The genus *Micronereis* was set up by Claparède (1863) for the species *M. variegata*. Its original definition—"Zwei tief getrennte Ruder am Füsstummel mit einem einzigen Rücken—und einem Bauchcirrus. Mundsegment borstenführend" was far from adequate, and later authors have added other diagnostic characters. The most complete and most recent definition is that of Fauvel (1923), but this requires slight modification in order to comprise the species which is the subject of this paper and which should unquestionably be placed in the same genus as *M. variegata*. The definition will then read as follows:

Genus *Micronereis* Claparède (char. emend.)

Body small. No tentacles. Palps rudimentary or absent. Proboscis without paragnaths. A pair of chitinous jaws. Four pairs of tentacular cirri. Prostomium and peristomium fused. Parapodia of first two setigers uniramous, the remainder biramous. Setae homogomph spinigers and, sometimes, smooth capillaries. Sexually dimorphic.

Micronereis nanaimoensis sp. n.

The following characters are common to both sexes. Body tapered posteriorly. Prostomium and peristomium fused and these, in turn, fused ventrally with the succeeding segment, but distinct dorsally. The head so formed rounded anteriorly, weakly bilobed, and bearing four pairs of fusiform tentacular cirri, the posterior pair the longest. Eyes large, the anterior pair the farther apart, with lenses, and considerably the larger (Figs. 1 and 2). Palps small, pointing downward. Two chitinous jaws, roughly triangular, with four or five teeth at the apex (Fig. 3). The segment immediately posterior to the head setigerous. The first two setigers with neuropodia only. The remainder with two very divergent rami terminally expanded and somewhat inflated, a subulate cirrus on each ramus (Figs. 4 and 5). The parapodia heavily ciliated throughout the body. The anus terminal with two red-brown cirri beneath it.

The following characters are peculiar to the male. Up to 13 mm. long and 23 setigers. Pygidium with a pair of lateral lobes between the cirri and the anus Fig. 6). Setae a mixture of homogomph spinigers and short fine capillaries (Fig. 4). Five or more specialized crested crotchets in the ventral ramus of the third setiger (Fig. 7). Coloration brilliant, a patterning of alternated straight and waved, transverse, orange-scarlet bars overlying a pale turquoise-blue ground, the tentacular cirri banded alternately red and white.

The following characters are peculiar to the female. Up to 15 mm. long, about 1 mm. wide at the anterior end excluding the parapodia, and 26 setigers. Pygidium a blunt cone, the anus slightly dorsal, no lateral lobes (Fig. 8). Setae all homogomph spinigers (Fig. 15). Coloration conspicuous, consisting of a covering of closely set, fine, transverse, dark red or red-brown lines on a cream ground, through which the green eggs shine in mature individuals. The tentacular cirri banded alternately cream and dark red.

M. nanaimoensis differs morphologically from *M. variegata* as described in the literature (Claparède, 1863; Racovitza, 1893; McIntosh, 1910; Fauvel, 1923) mainly in the following particulars:

SIZE. Females up to 15 mm. long and 26 setigers; males up to 13 mm. long and 23 setigers, Claparède, Racovitza, and Fauvel give 4 mm. long and 21 setigers in the former case and McIntosh and Fauvel give 2 mm. long and 17 to 18 setigers in the latter case for *M. variegata.*

PALPS. Quite definitely present, though small, Fauvel and McIntosh say they are absent in *M. variegata.*

JAWS. Identical in both sexes. Fauvel and Racovitza describe an extended bifurcate elongation in those of the males in *M. variegata.*

EGGS. Many hundreds of green eggs present in each jelly-mass. Racovitza refers to "about sixty" red eggs in *M. variegata.*

CRESTED CROTCHETS IN MALES. These are somewhat complex structures and five or more are present in each third neuropodium (Fig. 7). In *M. variegata* they

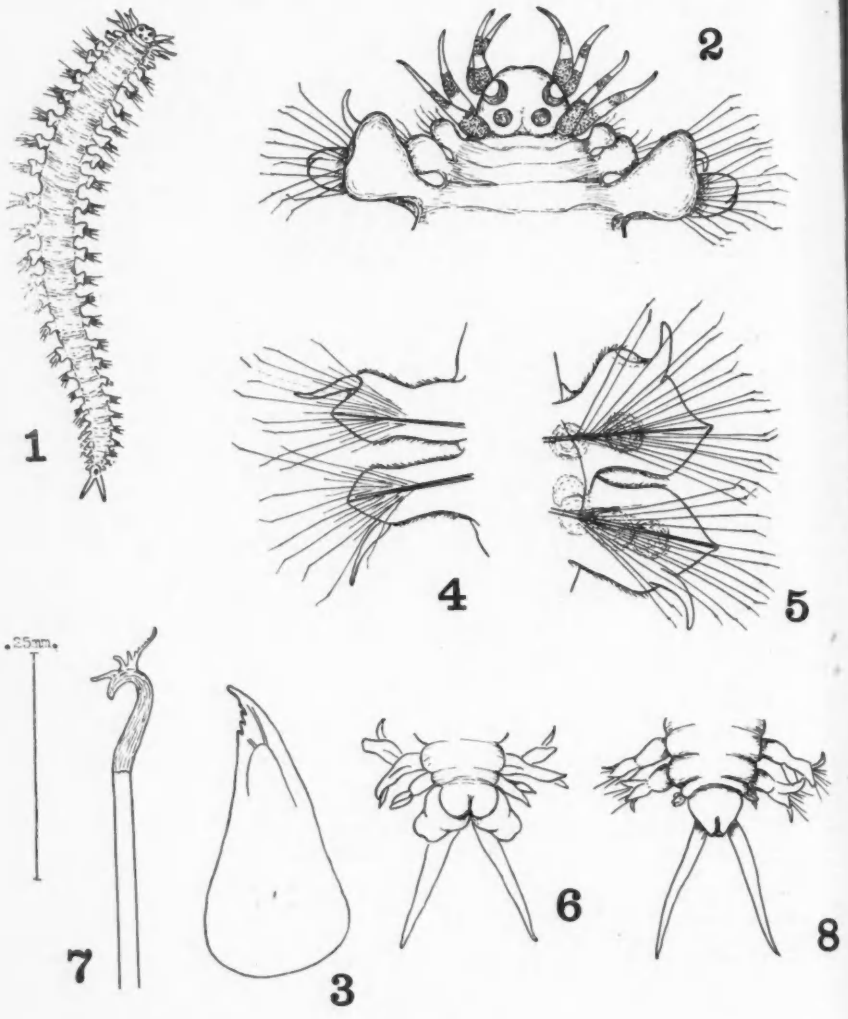

PLATE I. 1. *Miconereis nanaimoensis*. Dorsal view of female. 2. Anterior region of female. 3. Jaw. 4. Parapodium of male. 5. Parapodium of female, showing opening at base of notocirrus. 6. Anal end of male. Dorsal view. 7. Specialized neuropodial crotchet of male. 8. Anal end of female. Dorsal view.

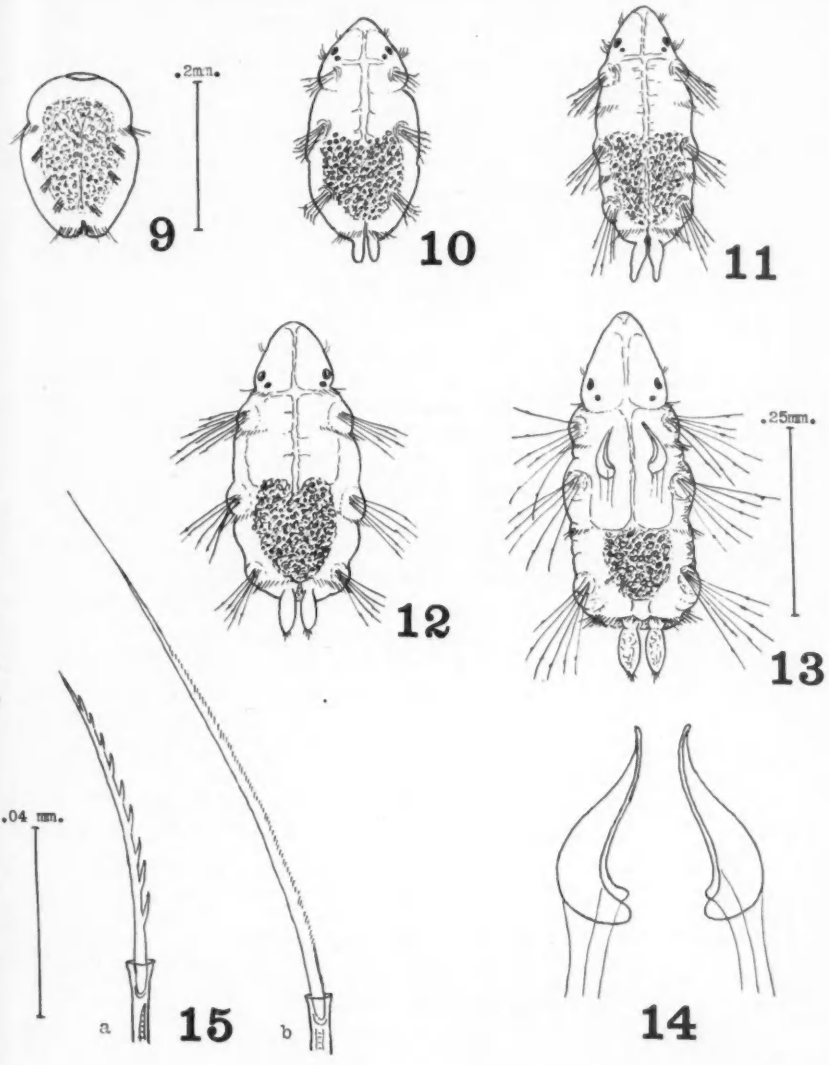

PLATE II. 9. Larva, 4 days old. 10. Larva, 6 days old. 11. Larva, 7 days old. 12. Larva, some days later than 11. 13. Larva, some days later than 12. 14. "Jaw-carriers" of larva at stage shown in 13. 15. Tips of setae: (*a*) of larva, (*b*) of adult.

are much simpler, as described by Racovitza and figured by Fauvel (1923, Fig. 128e), and only a pair occurs in each third neuropodium.

SETAE. In addition to homogomph spinigers simple capillaries are present in males. These are not described for *M. variegata.*

COLORATION. The general body-colour of *M. variegata* is described by Claparède as brown and by Fauvel as deep purple-red and no differentiation is made between the sexes (Claparède saw only a single female individual). In *M. nanaimoensis* the coloration is as described above and it differs considerably in the sexes. Both Claparède and Fauvel refer to a yellow coloration of the tentacular cirri in *M. variegata* which is absent in *M. nanaimoensis.*

JELLY-MASSES

The jelly-masses are usually attached to the vegetation on which they are found in such a way that a maximum amount of surface is exposed to the surrounding water. In these circumstances they are spherical or nearly so. The diameter may vary from about 10 mm. to 20 mm. and the sexually mature females within vary in length from 7 mm. to 15 mm. Occasionally two females have been found in one large jelly, but this is probably due to their settling near together and their jellies fusing. Only once have we found a male inside a jelly-mass with a female in nature, and he left it immediately the jelly-mass was disturbed. In captivity a male will occasionally enter a jelly-mass, but only very temporarily. The female, on the other hand, never leaves her jelly completely until all the larvae have vacated it and little remains but the hollow outermost layer. The eggs are gathered immediately after laying into a tight ball at the centre of the jelly-mass, the female curling herself around and over them. As development proceeds she secretes more jelly increasing the size of the mass and allowing expansion of the ball of eggs. By the time the larvae are developing setae and are capable of active movement they have been distributed uniformly through the jelly leaving enough space between them for the mother to pass and she continues constantly weaving her way amongst them until they are ready to swim free.

This behaviour of the female seems to be peculiar to *Micronereis* amongst the Nereidae though a similar care for the eggs occurs in some tubicolous species in which they are laid in the tube. Many species of polychaeta are known in which the eggs are laid in jelly-masses; Okuda (1946) gives an account of a number of such instances. In all these cases, however, the eggs are left to develop untended.

The earliest date on which we have found a jelly-mass is March 29th. This was a solitary example floating loose in a tide-pool. It contained eggs at an early stage of development which produced normal larvae in due course. They are fairly common on *Zostera* beds by the middle of April and, usually, none are to be found after the end of May. The single swarming we have observed took place on April 24th, but it is certain that another had occurred before this date since there were already jelly-masses containing well-advanced larvae at the time of

our observation and, as noted above, we had already found an isolated jelly-mass with developing eggs in March. It would be difficult to observe an early swarming since no tides sufficiently low to render the *Zostera* beds accessible usually occur in the early part of the year before April in this region, and only at night during the winter months.

SWARMING

No concentration of individuals at any one point over the *Zostera* or *Ulva* beds characterizes the swarming of *M. nanaimoensis*, neither sex is represented in appreciably greater quantity than the other, and the total number of swarmers is not large. There does not seem to be any structural modification incidental upon swarming, certainly nothing akin to that of a heteronereis form. The males can be differentiated from the females during swarming by their more rapid and more undulatory swimming and no attraction of the sexes to one another can be observed. We failed to find a single case of a female with the male attached by his specialized setae swimming over the *Zostera* beds, though subsequent observation of pairs in the laboratory convinced us that it must occur. Swarming is thus characterized solely by the facts that the participating individuals are sexually mature and free-swimming.

PAIRING AND EGG-LAYING

Proof that the individuals swimming with very rapid and undulatory movement were male was obtained by finding specialized hooks in the neuropodia of the third setiger and, having established the point in this way, they were readily distinguished subsequently by their vivid colouring. Preliminary experiments indicated that the males were not attracted to females which had already laid and were enclosed, with their eggs or larvae, in jelly-masses, but that, on the other hand, laying was stimulated in free mature females by the presence of the male. Two such females kept alone remained more or less quiescent on the bottom of the dish containing them and failed to lay during a period of observation extending over ten days. Two others into whose dish a male had been introduced behaved quite differently, swimming around quite actively and laying in the usual mass of jelly attached to the side of the dish after five and six days respectively.

Accordingly several pair of mature individuals were brought together and kept under observation. For some hours they swam around without there being any apparent attempt at close approach on the part of either sex, certainly without any discharge of sexual products by either. After some twenty-four hours, close approaches became increasingly frequent, but were followed by periods of separation. This went on for two or three days, after which contact was sustained and, finally, the male made efforts to attach himself by means of the hooked setae of the third setiger. He did not always succeed in doing so at once, the female dislodging him two or three times by vigorous wriggling. In his first efforts the male's point of attachment was more or less indiscriminate, sometimes near the

anterior and sometimes near the posterior end of the female, sometimes even laterally or more or less ventrally, but eventually he became firmly attached dorsally with his hooks inserted at, or very near, the twelfth setiger, his head pointing in the same direction as that of the female. In this position the pair remained comparatively quiescent for a few hours, after which they again separated. During the whole period of preliminary manoeuvring and attachment there was no evidence of sperm discharge, there was no clouding of the water and no spermatozoa could be found in it even in the immediate vicinity of the female. Since we have not witnessed the initiation of egg-laying we cannot be certain that the male was not again attached at the time this took place, but we are satisfied that no sperms were emitted until at least the majority of the eggs were laid. The male swam actively in the vicinity of the female whilst egg-laying was still in progress and visited her and the egg-mass, which were now covered with a thin film of jelly, at frequent intervals. At this stage sperms were discharged over and around the egg-mass and could be found in quantity in the jelly and in the water in the neighbourhood. These observations suggest that the presence of the male and his temporary attachment by means of his hooked setae stimulates the female to lay, but that it is the presence of the freshly laid eggs which in turn stimulate the male to the discharge of sperms.

The movement of the male is too rapid when he is swimming over the egg-mass to detect the emission of sperms, but on two occasions, streams of them could be seen emerging from the two lobes immediately above the anal cirri (Fig. 6) when he remained still. The length of the head of the sperms is about three μ. The diameter of the freshly laid egg is about 0.2 mm.

In order to determine how the eggs are set free it is necessary to observe the laying of the first few. This we have so far failed to do. A little later the body is so completely covered with eggs that it is impossible to follow closely what is occurring. The eggs seem to emerge simultaneously all along the body, there is no concentration of them at any one point such as would be expected to occur if they were freed through the perforations of the body-wall made by the male in inserting his hooked setae or if they emerged at the posterior end of the body as Racovitza (1894) believes to occur in the case of *M. variegata*. All that can be seen after laying is well established is the appearance of eggs at frequent intervals at the tips of the notopodia all along the body. They have not been seen actually emerging at that point, but the fact that in many cases parapodia of females, fixed before egg-laying was quite complete, show well defined openings at the base of the notocirri of a suitable size to admit of the passage of eggs suggests the probability that they do so (Fig. 5). On the other hand since the parapodia are kept in constant motion during laying, it is possible that the eggs emerge elsewhere and are transferred by the notopodia to the egg-mass. We hope to make further and more complete observations on this matter at the first opportunity. As soon as the eggs are all laid the female crawls free from the mass and over it, moulding it into a small ball surrounded with clear jelly.

Our observations differ from those of Racovitza (1893) on the pairing of *M. variegata* in that he found that the male remained attached to the female for

three days and left her immediately *after* egg-laying was complete. Racovitza, like ourselves, failed to determine how the eggs were discharged. His suggestion that it is through an orifice at the anal end of the body is based upon an experiment in which he compressed a ripe female and saw eggs emerging in that region, but this may well have been due to a rupture caused by the pressure.

DEVELOPMENT

The development of the eggs cannot be satisfactorily followed whilst they are still enclosed in the jelly-mass, but there is reason to think that periodical disturbance of the jelly to extract eggs and larvae for examination affects their development adversely. Certainly it is more irregular than in undisturbed jellies, many eggs developing much more slowly than others and emergence of the larvae from the jelly starting prematurely as standardized by that from undisturbed ones. Repeated rupture of the jelly-masses seems to interfere with its texture in such a way as to prevent the mother from circulating freely over her eggs whereby she keeps the developing larvae separated and, probably, aerated.

Figs. 9 to 13 illustrate stages of the development of larvae observed by extracting individuals periodically from a jelly-mass collected on *Zostera*. A sample of the eggs withdrawn from the jelly immediately after collection indicated that no division had yet taken place. They were spherical, green and about 0.2 mm. in diameter. Two days later ciliated larvae having one axis somewhat longer than the other had developed and were rotating slowly around the longer axis. Fig. 9 shows the condition after four days, Fig. 10 after six days, and Fig. 11 one day later. At this stage some larvae were swimming outside and around the jelly-mass, but they did not go far away from it and seemed to re-enter it at will. Thereafter increasing numbers of larvae left the jelly daily. Figs. 12 and 13 show somewhat later stages. After reaching that shown in Fig. 13 no further change has been seen to take place except that the red pigmentation throughout the body becomes more marked. The larvae are markedly positively heliotropic at this stage and any concentration of them on the illuminated side of the containing vessel is definitely red. The most striking development at this stage, however, is the appearance of what we have termed "jaw-carriers" (Fig. 14). No actual jaws, in the meaning of the toothed, chitinous structures found in the adults, are yet formed, but the "jaw-supports" occupy the position in the larval body in which the jaws might be expected to appear and they can be projected through the mouth like the chitinous jaws of an adult nereid. We suspect that at a later larval stage the jaws may develop at the apices of the "carriers". The larval setae are all homogomph spinigers as in female adults, but they are relatively coarser in the larvae (Fig. 15 a and b).

Thirteen days from the start of the observations a large majority of the larvae had left the jelly and a day later it was empty and the mother free. As compared with this, in a case of a similar jelly, but one left undisturbed, no larvae were seen to leave the jelly for ten days and it was not empty until sixteen days had elapsed. In each of these cases the jelly-masses were kept in sufficient water

to more than cover them in shallow dishes standing in running cold water, but no attempt was made to check or control the temperature and this may well be a factor in the rate of larval development.

Repeated attempts to rear larvae beyond the three-segmented stage illustrated in Fig. 13 have resulted in failure. They may remain pelagic for two or three weeks if no sand is present in the vessel containing them and the water is kept sufficiently cool, but eventually they settle to the bottom and die. This occurs whether a large portion of the water is changed daily or not and whether the water is aerated or not. If a layer of sand is present covering the bottom of the vessel a majority of the larvae settle out and bury themselves quickly, but, if the sand is disturbed, they readily emerge and resume swimming temporarily, and they survive no longer. The sand used in these experiments was taken from a *Zostera* bed on which *Micronereis* had spawned and seemed to contain ample organisms, both vegetable and animal, of a suitable size to supply necessary food for the larvae, but, to ensure this, a culture of mixed diatoms and small protozoa, obtained by keeping enriched, filtered sea-water in sunlight for some days, was added in some cases. In others the sand was heated over boiling water and cooled before adding the food material with the idea of destroying any possible organism inimical to the growth of the larvae which it might contain. All the experiments were carried out in shallow dishes standing in running cold water and kept in subdued light. Since no one of these courses succeeded in inducing the development of a fourth segment in the larvae, finely chopped *Ulva* and *Laminaria* was added to some of the cultures and, this failing, a number of animal foods were tried. Efforts have also been made to rear the larvae in running sea-water and in a plunger jar. So far, however, nothing has been found to induce the desired end. The longest period we were able to keep the larvae alive after their emergence from the jelly-mass was twenty-three days. In this case they were supplied with sand and chopped sea-weeds and about three-quarters of the water in the dish was renewed every second day.

These results seem to indicate that *M. nanaimoensis* is normally a bottom-living species shortly after it leaves the jelly-mass and that some factor yet to be determined is necessary for its growth beyond that stage. Our observations do not agree with those of Racovitza (1893) on the development of *M. variegata*. He refers to larvae with four pairs of parapodia in the jelly nine days after egg-laying and deduces that they were ready to leave the jelly since free larvae were found with five pairs of parapodia. Again, in discussing the evolution of the jaw, he refers to larvae with five to twelve pairs of parapodia. He does not, however, make it clear whether he reared the later larval stages or found them in their natural habitat.

COMPARISON OF THE DEVELOPMENT OF *M. NANAIMOENSIS* WITH THAT OF OTHER NEREIDAE

The characters of *Micronereis* diverge in so many particulars from those of other Nereidae that doubt has been expressed as to whether it should be included in that family (McIntosh, 1910). Ramsay (1914) summarized the morphological

arguments for so doing. Affinity with more typical Nereidae is shown on embryological grounds by the development of *M. nanaimoensis* so far as we have been able to follow it. On comparing, for instance, the stages shown in Figs. 9, 10 and 13 with those illustrated by Wilson (1932) for *N. pelagica* Linné and by Dales (1950) for *N. diversicolor* Müller, our Fig. 9 is comparable with Wilson's Fig. 1 and Dales's Fig. 4c; our Fig. 10 with Wilson's Fig. 3 and Dales's Fig. 4f; and our Fig. 13 with Wilson's Fig. 5 and Dales's Fig. 5b. The principle differences are in the last stage, when *N. pelagica* and *N. diversicolor* both show rudimentary tentacles and the latter rudimentary jaws. The jaws are said to develop in *N. pelagica* "a day or two after" the stage shown in Wilson's Fig. 5, that is, in about eleven days after fertilization. *M. nanaimoensis* larvae develop neither tentacles nor jaws for as long as we have been able to observe them, that is about thirty-four days from fertilization. The absence of tentacles was to have been anticipated since none are present in the adults; the development of what we have called "jaw-carriers" at the stage of Fig. 13 may perhaps, as we have suggested, be a forerunner of the production of jaws. Interesting differences occur in the intervals between fertilization and the attainment of the stages which we have compared in *N. diversicolor* and *M. nanaimoensis* respectively, the former taking about fourteen days to reach the stage shown by the latter in six days, and twenty-one days to arrive at the same stage as *M. nanaimoensis* reaches in ten or eleven. The periods for *N. pelagica* and *M. nanaimoensis* approximate, on the other hand, very nearly. Several days after the larvae of *N. pelagica* and *N. diversicolor* showed rudiments of a fourth setiger those of *M. nanaimoensis* failed to do so in our experiments, but this may have been due to the same factor in the conditions of rearing that eventually led to their death.

REFERENCES

Berkeley, E. Polychaetous Annelids from the Nanaimo district. Pt. 2. Phyllodocidae to Nereidae. *Contr. Canadian Biol.* (N.S.), **2**, 287–293, 1924.

Berkeley, E. and C. Polychaeta Errantia. *Canadian Pacific Fauna*, No. 9b(1), 1948.

Claparède, E. Beobachtungen über Anatomie und Entwicklungsgeschichte wirbellosen Thiere. Leipzig, 1863.

Dales, R. P. The reproduction and larval development of *Nereis diversicolor*, O. F. Müller. *J. Marine Biol. Assoc.*, **29**, 321–360, 1950.

Fauvel, P. Polychètes Errantes. *Faune de France*, No. 5, 1–488, 1923.

McIntosh, W. C. A monograph of the British Annelids. 2(2), Syllidae to Ariciidae, pp. 233–524, 1910.

Okuda, S. Studies on the development of Annelida Polychaeta, 1. *J. Fac. Sci. Hokkaido Imp. Univ.*, **9**(2), 115–219, 1946.

Racovitza, E. G. Sur la *Micronereis variegata* Clpd. *C. R. Acad. Sci. Paris*, **116**, 1390–1392, 1893.

Sur les amibocytes, l'ovogenèse et la ponte chez la *Micronereis variegata Clap. C.R. Acad. Sci. Paris*, **118**, 153–155, 1894.

Ramsay, L. N. G. On the Annelids of the Family Nereidae collected by Mr. F. A. Potts in the N.E. Pacific in 1911. *Proc. Zool. Soc. London*, pp. 237–250, 1914.

Wilson, D. P. The development of *Nereis pelagica* Linnaeus. *J. Marine Biol. Assoc.*, **18**, 203–217, 1932.